The Evaluation of Methods to Rapidly Assess Beverage Intake and Hydration Status

Samantha Kostelnik

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Brenda Davy, Chair
Kevin Davy
Valisa Hedrick
Travis Thomas

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ABSTRACT

Dehydration can impact the general population but it is particularly detrimental for athletes, due to their physical performance requirements. Although fluids in general contribute to meeting hydration needs, some beverages aid in the rehydration process more than others. The Beverage Intake Questionnaire (BEVQ-15) is a food frequency questionnaire (FFQ) that can rapidly assess habitual beverage intake; this FFQ has been validated in children and adults. However, no beverage consumption questionnaire has been validated in athletes. In addition to monitoring fluid intake, hydration status can be assessed through urinary and blood indices. Urine color (UC) has been utilized as a practical hydration biomarker in several populations. However, this biomarker has not been validated among the general population of collegiate athletes. The first study (n=58): formulated a novel whey-permeate-based beverage to promote hydration and assess its sensory characteristics in the general population. The overall acceptability of the beverage was lower than the control beverage, according to a 9-point Likert scale ($\bar{x}$ = 4.5 – 4.9 and $\bar{x}$ = 6.7, respectively). The second study (n=120): evaluated the comparative validity and reliability of the BEVQ-15 and UC within NCAA Division 1 collegiate athletes. Associations were noted between the BEVQ-15 and multiple 24-hr dietary recalls (reference method) for total beverage fl oz and kcal (r=0.41 and r=0.47, $p \leq 0.05$, respectively). There were associations between athlete's UC and urinary specific gravity (USG; hydration biomarker) rated by athletes and researchers (r=0.67 and 0.88, $p \leq 0.05$, respectively). Lastly, a systematic review was performed to evaluate original research addressing the validity of UC as a hydration biomarker in the adult population more broadly, including athletes and older adults. Eleven of 424 articles met inclusion criteria, and the available research generally reported significant correlations between UC and other hydration indices (r=0.35-0.93). However, limitations in existing research were evident. Although the BEVQ-15 may be a valid beverage intake assessment method in collegiate athletes, additional modifications were identified which could improve its validity. Future work includes re-evaluating the validity and reliability of the BEVQ-15 specifically modified for athletes, as well as assessing the sensitivity of this FFQ to detect changes in beverage intake.

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GENERAL AUDIENCE ABSTRACT

Drinking adequate amounts of fluids is important for maintaining normal bodily functions. When body water losses exceed fluid intake, dehydration may result, which can lead to numerous consequences such as headaches, dizziness, decreased mental focus, and fatigue. An athlete, who has high physical demands, may experience these negative consequences as well as muscle cramps, increased strain on the heart, and decreased athletic performance. Some beverages can replenish lost fluids better than others, due to their electrolyte (i.e. sodium, potassium, magnesium) content. This may include whey-permeate based beverages. In order to prevent dehydration, it is important to monitor fluid consumption and fluid losses. A beverage intake questionnaire (BEVQ-15) can be used to quickly assess usual beverage intake. Studies have shown that this questionnaire is accurate in children, adolescents, and adults. However, there are currently no validated methods for usual habitual beverage intake in athletes. This dissertation evaluated the taste of a new whey-permeate hydration beverage, and the accuracy and test-retest reliability of the BEVQ-15 within NCAA Division 1 collegiate athletes and found positive results. Measurements in urine and blood can be also be used to assess hydration status, but some of these methods are more expensive and less practical for daily use in real-world settings. Urine color (UC) has been studied as a hydration indicator, but this dissertation is the first to evaluate the accuracy and reliability of this method within a diverse group of collegiate athletes, in a real-world setting. Our results suggest that UC is a simple and reasonably accurate hydration assessment method when compared to another urinary assessment method. Nonetheless, there is limited research which has studied this topic. Future work can address methods to improve the effectiveness of these approaches for maintaining and evaluating fluid intake and status in the collegiate athletic population.

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CHAPTER 2

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CHAPTER 3

This manuscript is in preparation for submission to a scientific journal. Preliminary data from this study has been presented at the American College of Sports Medicine Annual conference (2019). Dr. Brenda Davy, Kevin Davy, PhD, Valisa Hedrick, PhD RD, and Michelle Rockwell, PhD, Human Nutrition, Foods, and Exercise, Virginia Tech participated in study design, data interpretation, and manuscript edits. Travis Thomas, PhD, RDN, CSSD, LD, FAND, Department of Athletic Training & Clinical Nutrition, University of Kentucky participated in manuscript edits.

CHAPTER 4

This manuscript is under review for submission to a scientific journal. Drs. Brenda Davy, Kevin Davy, Valisa Hedrick and Travis Thomas assisted with designing the review protocol and data interpretation, provided critical feedback on the report, contributed to writing the report, and approved the final version of the manuscript. Dr. Brenda Davy, supervised the development of the protocol and report, and screened articles for quality.

CHAPTER 1: INTRODUCTION

BACKGROUND

Water plays numerous roles within the body, including thermoregulation, cellular homeostasis, transporting nutrients throughout the circulatory system, and eliminating waste and other toxins through the renal system or sweat.[1,2] Adequate fluid consumption is crucial for the well-being of the human body and the ability to sustain a euhydrated, healthy state. Euhydration is defined as having normal body water content.[3] Dehydration is defined as the act of losing body water. Hypohydration, on the other hand, is when the body is in a water deficit.[4] Mild dehydration can occur when there is a loss of 1-2% body water and can lead to a decrease in concentration, memory and an increase in anxiety.[4] Severe hypohydration occurs when there is a loss of 2-4% body weight of water, specifically during periods of prolonged sweat loss.[1,5,6] Adverse physiological consequences of hypohydration include fatigue, muscle cramps, increased cardiovascular (CV) strain, impaired cognitive functioning, increased resting metabolic rate (RMR), and dyspnea.[1,7-9]

In athletes, dehydration can impair performance, and also result in acute renal failure, exertional heat illness, vomiting, and a decrease in alertness.[3,10] The prevalence of dehydration among athletes is common and athletes are often dehydrated when they begin exercising.[6] In fact, Hew-Butler et al., found 27%-55% of the athletes to be classified as dehydrated, based on urinary indices.[11]

TOTAL BODY WATER

Fluid Regulation

A major constituent of the body is water, which accounts for about 75% of total body weight at birth but decreases by age to around 50%.[1,2] Additionally, the amount of adipose tissue can affect total body water (TBW). A lean body will contain between 60-70% water, while an obese body will contain 45-55% water.[12] Water within the body remains fairly constant as it moves through the intracellular and extracellular fluid compartments via a process called osmosis.[12] In order to maintain water balance, the sodium-potassium adenosine triphosphatase pump (Na/K-ATPase Pump) assists in distributing sodium and potassium ions between the major compartments of each cell.[12] This equilibrium is also regulated by numerous hormones such as cortisone, angiotensin II, and antidiuretic hormone.[12,13] In times of dehydration, the body will experience hypovolemia (low blood volume), causing antidiuretic hormone to release a signal, from the posterior pituitary, that results in the conservation of water within the kidneys.[12-14] Simultaneously, the angiotensin II hormone is activated and induces the sensation of thirst.[12,14,15] Although 'drink to thirst' is recommended, some have suggested that thirst is not the best indicator of hydration status.[16,17] During times of hyperhydration, or drinking in excess of fluid needs, the intracellular and extracellular fluids will expand and the body will produce urine. This could pose inconveniences for athletes because over-consuming liquids will result in an increased need to urinate. Additionally, the excess fluid within the body can dilute plasma sodium, causing an increased risk of hyponatremia, which can lead to dilution encephalopathy, edema and even death.[3] Sodium within the body helps maintain cellular homeostasis and fluid regulation.[18]

WATER/FLUID INTAKE RECOMMENDATIONS

Recommendations for General Population

According to the National Academy of Medicine (NAM), the adequate intake (AI) of water is different for males and females and also depends on age. AI is a recommended intake based on estimations of nutrient intake when the Recommended Dietary Intake cannot be determined.[19] The AI for plain water is different between males and females who are between the ages of 19 and 70+ years old (3.7 L/day and 2.7 L/day, respectively).[20] However, these recommendations do not take physical activity, health status, or environmental factors into account.[21] Additionally, fluids can be distributed differently throughout the day, depending on body composition and dietary consumption.[22] In fact, beverages contribute approximately 80% of our total fluid intake but fluids can also be obtained through solid foods (e.g. fruits).[20]

The Beverage Hydration Index (BHI) was developed to quantify beverages, based on the hydration response through the accumulation of urine that is excreted at the end of every hour throughout the study session following ingestion of 1 L of a test beverage.[22] This urine collection is then compared to the standard excretion following the consumption of 500 ml of plain water, that was consumed within 15 minutes. Each beverage is assigned a value between 0 – 2.5 is given to a beverage. A BHI of 1.0 was standardized for water; therefore, a value > 1.0 indicates greater fluid retention and a value < 1.0 indicates less fluid retention.[22,23] Maughan et al. reported that beverages with higher electrolyte and macronutrient contents (e.g. carbohydrates) also had a higher BHI than plain water.[22] For example, a by-product of dairy products (i.e. Greek yogurt and many cheeses) called whey-permeate is rich with electrolytes and whey proteins.[24] Therefore, beverages containing whey-permeate may have a higher BHI. Moreover, the ability to sustain adequate fluid balance is positively associated in beverages with a higher BHI.[22] Clarke et al., found that beverages containing amino acids promote a positive fluid balance in older adults, as determined by the BHI.[23] Therefore, using this tool could promote specific beverage intake recommendations for the general population, especially those beverages that will assist in maintaining euhydration.

Recommendations for Athletic Population

Before Exercise

An athlete is defined as someone who is capable of utilizing stamina, physical strength and agility in order to performed the exercise or sport, in which they have proper training.[25] For optimal performance and to maximize the absorption of fluids, an athlete should begin consuming fluids several hours prior to performing physical activity.[3,10,26] In fact, a euhydrated state at the onset of exercise and allowing the body's hydration levels to return to normal, based on urine color, will allow the athlete to perform at a higher level.[26] Therefore, approximately 2-4 hours prior to exercise, an athlete should consume between 5-10 mL/kg of body weight of fluids.[26] The inclusion of sodium, either in a beverage or in a snack before exercise, will further increase the retention of fluids.[3,20,26]

During Exercise

During exercise, athletes lose heat and water through sweat evaporation, which could result in dehydration.[3] Studies have shown that to prevent dehydration during exercise, about 0.4-0.8 L/hr of fluid should be consumed.[26] Depending on the duration of the exercise bout, a combination of fluids (i.e. plain water and those including electrolytes) should be consumed.[3] However, sweat rates can vary between athletes and there is no single standardized hydration plan.[3,27] In fact, sweat rates can range from 0.29 – 2.60 L/hr, depending on the sport, training condition, and gender.[3] Therefore, it is important for each athlete to develop a hydration plan that will accommodate their individual characteristics, such as exercise type and duration, sweat rate and concentration, and the environment in which they are exercising.[3,27] It is also recommended that athletes monitor their changes in body weight, in order to calculate sweat loss.[3]

After Exercise

It is important for athletes to replace the lost fluids within 2 hours of exercising.[10] The American College of Sports Medicine (ACSM) recommends that each athlete should consume approximately 1.25 - 1.5 L of fluid per kilogram of body weight lost in order to efficiently recover following exercise.[3,26] Proper rehydration fluids include water, and beverages with electrolytes and carbohydrate in order to replenish fluid and electrolyte stores.[10] More specifically, beverages with a concentration of 100 mmol/L of sodium are more effective at promoting rehydration than beverages with a lower sodium concentration.[28] Additionally, foods that contain sodium should also be consumed, after exercise, to aid in fluid retention.[3]

BEVERAGE CONSUMPTION HABITS

Beverage Consumption in the General Population

The consumption of beverages, particularly those that are nutrient-rich like water and milk, are critical to maintaining adequate hydration status. Water alone is essential for the cells, organs, and tissues within the body to properly function every day.[1] Regardless of the amount of energy exerted, individuals will become dehydrated and cannot survive without an adequate intake of fluids. Inadequate hydration can cause impairments in cognitive, gastrointestinal, and cardiovascular functioning, as well as physical performance.[1,29] In addition to these impairments, studies have shown that being inadequately hydrated is associated with weight status, particularly in individuals with higher body mass index (BMI).[30] Due to the faster metabolic rate and higher body weight, obese and overweight individuals have greater fluid needs.[30] With an increase in obesity in the country, there has been a significant increase in sugar sweetened beverage (SSB) consumption over the past 30 years, which have been shown to be associated with numerous adverse health effects.[31,32]

In 2012, Ogden et al., reported that close to 50% of the youth in the United States were either overweight or obese.[33] The food and beverage consumption patterns seen within young children are contributing to their excess body weight. Additionally, these patterns often reflect those of their parental figures or can be altered due to environmental exposures.[34] For instance, lower income families, who are on food assistance programs, tend to eat and drink food products that are unhealthy due to their convenience and low costs.[35]

A little over 50% of the U.S. adult population, according to the 2011-2014 Centers for Disease Control and Prevention, are consuming at least one SSB per day, with a higher percentage in males (54%).[36] However, based upon National Health and Nutrition Examination Survey (NHANES) data from 2009-2012, adult men are also consuming more water than adult women per day (3.46 L and 2.75 L, respectively). These values are on the cusp of the AI of water for both sexes. Hispanic men and women (3.33 L and 2.58 L, respectively) consume less daily water than non-Hispanic white men and women, but they consume more than non-Hispanic black men and women (2.92 L and 2.41 L, respectively).[37]

Beverage Intake Assessment

With a need to reduce the risk of developing chronic diseases (i.e. obesity, diabetes, hypertension, etc.), researchers have examined beverage consumption patterns in different populations (see Table 1.1). The Beverage Intake Questionnaire (BEVQ-15) was developed by our research group to rapidly and quantitatively measure habitual beverage consumption over the past 30 days.[38] This questionnaire consists of fifteen beverage categories and an “other” category that is available for the beverages that are not listed.[38,39] Studies that have used this questionnaire, in an adult population, have reported validity (r = 0.34-0.98), when compared to multiple dietary recalls using the Multiple Pass Method, and test-retest reliability (r = 0.61-0.94). This food frequency questionnaire (FFQ) has been modified and validated in different populations. For example, Elwan et al studied individuals living in a rural Alaskan village with the goal of measuring beverage consumption and attitudes.[40] The researchers modified the BEVQ-15 by removing four questions on alcohol and tea/coffee consumption, resulting in an 11 category questionnaire. The researchers found that this particular group was consuming higher amounts of SSBs (100% children/adolescents and 72% adults in village vs 66% children and

61% adults in US) and insufficient amounts of water and milk (890 ± 483 mL and 250 ± 90 mL, respectively).[40]

A recent study investigated the validity and reliability of the BEVQ in children and adolescents in the New River Valley, Virginia. This study compared the BEVQ with multiple 24 hr dietary recalls. These recalls were conducted by a trained research assistant who utilized the multiple pass method. The research team found that there was no significant difference between the two methods for milk and total beverage calories for children, and no difference for water (g) and SSB calories in adolescents. Therefore, the BEVQ appears to be a valid and reliable method to measure habitual beverage intake in these populations.[41] African American and Hispanic children between the ages of 2 and 5 years old may have unique beverage consumption patterns. A study conducted by Lora et al., included a group of preschool aged African American and Hispanic children and measured their beverage consumption habits using a modified version of the BEVQ-15.[35,42] Due to the ages of the children in both of these studies, either the mother or father completed a version of the BEVQ for their child.[35,42] However, limited health literacy of the parents in these minority groups could also have impacted the BEVQ results.

Close to one-third of the United States population demonstrates low health literacy skills.[43] This is a critical issue that, according to Yin et al., is associated with negative health outcomes and an increase in health care costs. [43,44] With the increase in computerized tools over the past decade, our research group created an interactive multimedia version of the BEVQ (IMM-BEVQ), (see Table 1.1), to compare its reliability to a paper-administered version among individuals with low health literacy.[44,45] Using the IMM-BEVQ, the research team added an option to have the questionnaire read aloud to the subjects, had separate pages for each of the beverage categories and provided visual aids of each beverage and the different portion sizes available.[44] Though the IMM-BEVQ had many advantages and was able to determine changes in beverages intake patterns, the individuals who were low in health literacy were still challenged.[44] Nonetheless, the results demonstrated that those who were less knowledgeable of health related topics tended to consume greater amounts of SSB (21 ± 33 kcal) and total beverage calories (65 ± 49 kcal) than those with adequate health literacy.[45]

Beverage Consumption in the Athletic Population

Adequate hydration is crucial for optimizing athletic performance. A dehydrated athlete will experience the previously mentioned negative outcomes, as well as a decrease in muscular strength, endurance and power.[1,46] In order to avoid dehydration, athletes should consume fluids throughout the day but primarily focus on pre-practice fluid consumption.[47] Athletes who train outdoors, especially during the summer months (i.e. football, soccer, tennis, etc.), are more prone to heat related injuries when not properly hydrated prior to exerting energy.[48,49] While the previous recommendations are provided for athletes in general, athletes should develop an individualized hydration plan.[16] However, overconsuming fluids can lead to fluid intoxication, which occurs when beverage intake exceeds excretion to the point of hyponatremia.[12,50]

Athletes lose sweat electrolytes as they train; however, this loss is dependent on several factors, including the individual, the type and duration of training, and the environment.[3] Following exercise, it is crucial for an athlete to replace the fluids and nutrients (i.e. carbohydrates) lost in order to rebuild glycogen stores and efficiently rehydrate the body.[10] To maximize fluid retention, beverages which contain electrolytes (i.e. sodium and potassium) and other macronutrients, such as carbohydrates.[51,52] The inclusion of these solutes may lead to increased intestinal water uptake, and a reduced rate at which gastric contents are emptied.[53,54,55] Therefore, in order to avoid hypohydration and facilitate recovery, athletes should include foods and beverages that are rich in electrolytes.[3] However, there are currently no validated methods or food frequency questionnaires that can be used to assess habitual beverage consumption in athletes.

Table 1.1 Validation Studies of Beverage Intake Assessment Tools

Author	Publication Year	Target Population	Sample Size	Assessment Tool	Findings	Limitations
Laja-Garcia AI, Samaniego Vaesken ML1, Partearroyo T1, Varela Moreiras G.[56]	2019	18-39 yo, Spanish Adults	40	HSQ (Hydration Status Questionnaire)	The HSQ mainly evaluated water intake (WI), water elimination (WE), water balance (WB). WB and WI were moderately correlated with urinary specific gravity (r=-0.524) and urine color (r=-0.392). The Bland Altman plots demonstrated an acceptable agreement between the measures.	Body water content was assessed through bioelectrical impedance (BIA). Blood parameters at baseline were not known; therefore, changes in total body water were due to changes in plasma volume.
Marakis G., Kontopoulou L., Garofalakis G., et al.[57]	2019	20-23 yo, Greek young adults	59	DFQ (Drinks Frequency Questionnaire)	No significant differences between the DFQ and a 7 day weighed food record diary for total drink intake. Strong positive correlations for milk and coffee/tea (r=461-902). Bland Altman plots indicated an agreement between the two methods.	Small sample size and the results cannot be generalizable. Under-reporting error due to self-reporting. A 7 day weighed food record diary is burdensome on participants.
Vanderlee, L., Reid JL., White, CM., et al.[58]	2018	16-30 yo, Canadian young adults	50	BFQ (Beverage Frequency Questionnaire)	Most of the beverage categories presented significant positive correlations (r= 0.18 – 0.92) between the BFQ and a 7-day food record. There was also a strong agreement between these methods.	Under-reporting due to self-reporting. There was not biomarker used to assess sugar intake in order to better capture data on certain beverage categories.
Hedrick VE, Myers EA, Zoellner JM, Duffey KJ, Davy BM[59]	2018	$\geq$18 yo, US Adults	404	HBI-Q (Healthy Beverage Index Questionnaire)	No significant differences between individual HBI scores. However, the correlation for total HBI scores between HBI-Q and dietary recalls was significant (r=0.96). Bland-Altman analysis presented 92% agreement between HBI-Q scores and	Under-reporting error occurred due to self-reporting. Estimated energy needs were calculated to determine HBI scores for the HBI-Q

					dietary recall. This method is less time consuming than 24 hr recalls.	
De Cock N, Van Camp J, Kolsteren P, et al.[60]	2016	14-16 yo, European adolescents	179 (reliability) and 99 (validity)	Beverage FFQ (Beverage Food Frequency Questionnaire)	The reliability and validity of this snack and beverage FFQ were acceptable for the target population and can be used to assess disease-diet relationships and intervention effects. The reliability study presented r=0.68 and r=0.71 for beverages (healthy and unhealthy) consumed per day against the 24 hr recalls. The validity study presented r=0.17 and r=0.69 for beverages (healthy and unhealthy) consumed per day against the 24 hr recalls.	The sample population might not be generalizable to other populations. The items selected for the FFQ were based on the consumption habits of adolescents. The 24 hr recalls relied on memory. Possible spillover effects on eating behaviors.
Ferreira-Pego C, Nissensohn M, Kavouras SA, et al.[61]	2016	55-75 yo, Spanish Adults	160	Spanish Beverage Intake Questionnaire	The Bland-Altman plots showed an acceptable agreement between total daily fluid intake using the questionnaire and urine osmolality and 24h urine volume (R^2=0.20). No differences found between baseline and 6 months or 1-year assessment for the type of beverage or total daily fluid intake.	Serving sizes may have caused beverage category underestimation. The population was only in middle-aged and elderly adults. There was no method for assuring the completeness of the 24 hr urine sample. The use of frozen urine samples.
Hooper LP, Myers EA, Zoellner JM, Davy BM, Hedrick VE.[44]	2016	$\geq$18 yo, US Adults	273	IMM-BEVQ-15 (Interactive Multimedia Beverage Intake Questionnaire with 15 categories)	Lower HL group fell below the correlation range for validation studies (0.4-0.7). Means for intake of SSB and total beverage consumption were significantly different between IMM-BEVQ and diet recalls at baseline for all participants. All beverage categories were significantly correlated between IMM-BEVQ1 and diet recalls (r=0.17 to 0.78).	Participants consumed a minimum of 200 kcals/day of SSB to be eligible. There was a lack of participant diversity. Under-reporting error occurred due to self-reporting food intake records (FIR). The low health literacy (HL) individuals may need assistance in order to compare across all HL levels.

Karabudak E, Koksal E.[62]	2016	19-55 yo, Turkish Adults	291	BIQ (Beverage Intake Questionnaire)	The BIQ and DIR were significantly correlated for all beverages except for alcoholic beverages. There was a significant correlation between BIQ1 and BIQ2 for total fluid intake (r=0.84). Therefore, this tool is useful for assessing habitual beverage intake consumption patterns to evaluate hydration status for adults.	Participants completed the self-administered BIQ and dietary intake recall which could have caused underestimations. In the Turkish society, it is not common to consume alcoholic beverages in high amounts or frequencies therefore lead to the amount in the dietary intake recalls to be less than in the BIQ.
Koleilat M, Whaley SE.[63]	2016	2-4 yo, English and Spanish Children	70	CFBIQ (Child Food and Beverage Intake Questionnaire)	Intraclass correlations for CFBIQ ranged from 0.48 to 0.87. Correlations ranged from r=0.15 to r=0.59 for beverages. The CFBIQ exhibited weak to moderate test-retest reliability and moderate to strong validity in foods and beverages.	Generalizability may be limited because the sample comprised of 2-4 year olds. Self-reporting could have caused underestimating portion sizes.
Lora KR, Davy B, Hedrick V, Ferris AM, Anderson MP, Wakefield D.[42]	2016	3-5 yo, Hispanic Children	109	BEVQ-PS (Beverage Intake Questionnaire Pre-School)	Validation correlation coefficients ranged from r=0.20-0.37. The test-retest reliability correlation coefficients ranged from r=0.20-0.68 due to low frequency of consumption. Mean differences between BEVQ-PS and FIR for certain categories were not significantly different but were significantly correlated.	Recruitment was only at day-care centers. The literacy level of the participants may have yielded a low validity.
Riebl SK, Paone AC, Hedrick VE, Zoellner JM, Estabrooks PA, Davy BM.[45]	2013	$\geq$21 yo, US Adults	60	IMM-BEVQ-15 (Interactive Multimedia Beverage Intake Questionnaire with 15 categories)	All beverage categories from IMM-1 and paper versions were correlated (r=0.34-0.98). SSB and total beverage gram and kcal responses were correlated between each beverage questionnaire version (r=0.92-0.95).	Under-reporting error occurred due to self-reporting FIR. Duration between questionnaire administration caused possible participant bias.

Hedrick VE, Savla J, Comber DL, et al.[38]	2012	≥18 yo, US Adults	1596	BEVQ-15 (Beverage Intake Questionnaire with 15 categories)	The BEVQ-19 was reduced to a 15 category questionnaire to assess habitual beverage intake. The BEVQ-15 was more correlated with three 24 hr recalls with R^2=0.69 for SSB and R^2 = 0.59 for total beverage energy. This questionnaire is suitable for measuring a range of adult populations.	There was a high percentage of women who initially participated. The sample size was very large and primarily overweight or obese. Future work is needed to detect changes in beverage intake and determine whether this tool could be used for interventions. The data is subjective due to self-reporting.
Hedrick VE, Comber DL, Estabrooks PA, Savla J, Davy BM[39]	2010	≥21 yo, US Adults	150	BEVQ-19 (Beverage Intake Questionnaire with 19 categories)	Correlations for water, SSB and total beverages ranged from r=0.46-0.53 between the BEVQ1 and the FIR. Reliability was acceptable (r=0.45-0.87) as FFQs as compared to the typical correlations of 0.5-0.7.	Underestimation of beverage categories due to upper limits. The beverage category descriptions were unclear at times for many participants. Under-reporting error is possible due to self-reporting FIR.
Muckelbauer R, Libuda L, Kersting M.[64]	2010	7-9 yo, German Children	35	24 hr RQ (24 hour Recall Questionnaire)	Children could recall the beverage categories consumed. The correlation coefficient for a single beverage category, between the RQ and WR, were higher (r=0.86-0.91) than the coefficient for total 24h beverage volume (r=0.72). Additionally, the children were able to differentiate certain beverages but this may depend on country-specific consumption habits.	Validity was assessed at individual home based level, but the RQ was developed for use at the school group. The parental report of their child's dietary intake was the reference method. The study had a small sample size and prone to selection bias. The exact nutrient and energy intake from beverages cannot be assessed through this questionnaire.

Marshall TA, Eichenberger Gilmore JM, Broffitt B, Levy SM, Stumbo PJ.[65]	2003	6 month-5 yo, US Children	240	Beverage FQ (Beverage Frequency Questionnaire)	Correlations between the FQ and dietary diaries were stronger for all subjects rather than just beverage consumers alone. Mean daily beverage consumption correlations ranged from r=0.95-0.99. The quantitative frequency questionnaire can provide a relative estimate of beverage, vitamin D, and calcium intake.	Questionnaire and Food diary were mailed to the parents at the same time. Human milk intake was estimated using different formulas for the questionnaire and diary. Juice drinks and 100% juice were difficult to distinguish. Generalizability may be limited because parents were primarily white and well educated.

HYDRATION STATUS ASSESSMENT TOOLS

Assessment of Hydration in Athletes

There are numerous methods to measure an individual's hydration status, including urine osmolality (Uosm), plasma/serum osmolality, changes in body weight, urine specific gravity (USG), urine volume and urine color.[3,24,52] Osmolality is defined as the concentration of solutes in a solution.[49,66] Of those assessment strategies listed, the gold standard for measuring hydration status is plasma osmolality.[49] Total body water and plasma osmolality are the most precise in measuring hydration status because they can detect acute changes in hydration status.[3] However, this method is more expensive and less feasible when conducting large scale studies.[3,67] Urine volume and urine color (UC) are less precise but have been validated (summarized in Table 1.2), as practical biomarkers of hydration status.[68] A practical biomarker is one that is does not require technical expertise, is cost effective, and can be completed in a timely manner and in real-world settings.[3] The validation of UC, as a hydration biomarker is still uncertain for the general adult population due to limited research in this area. To date, no systematic reviews have evaluated the validity of UC as a hydration biomarker in the general adult population, including athletes.

Table 1.2. Validation Studies of Hydration Biomarker Tools

Author	Publication Year	Target Population	Sample Size	Hydration Biomarker	Findings	Limitations
Hew-Butler TD., Eskin C., Bickman J., et al.[11]	2018	US NCAA Division 1 Collegiate Athletes of any age	40	Blood vs Urine Indices for assessing Hydration	Serum Sodium demonstrated a significantly positive relationship with Uosm ($r=0.18$; $p<0.01$). Serum Sodium had a significant correlation with thirst rating ($r=0.12$; $p<0.001$). Serum Sodium had a significant correlation with serum osmolality ($r=0.20$; $p<0.001$). Uosm had a significant correlation with USG ($r=0.64$); $p<0.001$).	Inability to control fluid intake or standardize exercise before testing.
Kavouras, SA., Johnson, EC., Bougatsas, D., et al.[69]	2016	8-14 yo, Greek Children	210	UC	Urine color and urine osmolality had a strong positive relationship (R^2 = 0.45, $p<0.001$). Urine color is an applicable diagnostic tool to assess dehydration in children.	A 24-hour urine sample is an impractical method. The first morning void is susceptible to error and presents a higher estimation of dehydration.
Sommerfield, LM., McAnulty SR., McBride, JM., et al.[70]	2016	US NCAA Division 1 Collegiate Athletes of any age	56 Males 26 Females	USG	No significant correlations between USG and plasma osmolality in hydration status for males or females. Research team concluded to use caution when using USG to measure hydration status in collegiate athletes.	There was a low specificity of USG could be inaccurately classifying athletes as dehydrated.
Fernandez-Elias VE., Martinez-Abellan A., Lopez-Gullon JM, et al.[71]	2014	Athletes of Olympic combat sports, of any age	345	Uosm, USG, UC, BIA, TPS	High correlation ($r=0.89$; $p=0.000$) between Uosm and USG; however, the relationship weakened as dehydration increased. Urine color was significantly correlated with Uosm when comparing the whole sample ($r=0.743$; $p=0.000$). TPS had a low but significant correlation with Uosm ($r<0.315$; $p<0.05$). No	Used segmental BIA but mono-frequency analysis.

					significance between BIA and Uosm.	
Armstrong LE., Maresh CM., Castellani JW., et al.[68]	1994	US College students and NCAA Collegiate athletes, of any age	54	Urinary Indices	Uosm and USG were significantly correlated ($r=0.97$; $p<0.001$). Urine color and USG were significantly correlated ($r=0.80$; $p<0.001$). Urine color and Uosm were significantly correlated ($r=0.82$; $p<0.001$).	Low sample of women (15%). The only sport used for assessment was tennis. Small sample size and the geographical region may not be generalizable to the rest of the country.

URINARY BIOMARKERS

Urinary Specific Gravity

USG is the concentration of solutes in the urine and can be measured using a urine dip strip or with refractometry.[67] Specifically, a refractometer is more simple and precise in assessing USG, such as measuring UC. However, this tool can be more expensive than using other methods.[10] According to Armstrong, et al., a USG value in the non-athletic population of $\leq$ 1.013 is considered hyper-hydrated, 1.013-1.029 is considered euhydrated, and $\geq$ 1.029 is considered dehydrated.[68] However, the National Athletic Trainer's Association (NATA) considers that a USG value between 1.010 - 1.020 as dehydration for athletes.[10] In order to obtain the most accurate measurement of hydration status, the first morning void or a sample taken after hours of stable hydration will allow researchers to determine euhydration vs dehydration.[3] Other researchers report that the second morning void is more accurate; however, there is no research that has tested which approach is more valid.[68]

Urine Color

The byproduct of the breakdown of hemoglobin, known as urochrome, gives urine its color.[69] The greater the concentration of urochrome, the darker the color of urine.[69] This hydration biomarker is inexpensive and can easily be self-assessed by athletes. Armstrong, et al., developed an eight scale urine color chart that an individual can use to assess their hydration status by comparing their own urine to the color chart. The chart ranges from very pale yellow to dark brownish-green. Ideally, an athlete should provide “very pale yellow/pale yellow” urine, which indicates euhydration.[68] Any color darker than “pale yellow” indicates dehydration or hypohydration. However, vitamin supplements and medications can cause a change in urine color, resulting in a misrepresentation of hydration status.[72] Additionally, certain foods can alter urine color including coffee, beets, asparagus, carrots, and more.[73] The most effective way of measuring urine color is to collect a midstream sample to compare to the color chart. This prevents the dilution of urine in toilet water.[26,68]

CONCLUSION

Numerous beverage and hydration assessment tools have been developed and their validity investigated in different populations. Table 1.1 presented a summary of validated beverage consumption tools with significant findings and limitations. Table 1.2 presented a summary of validated hydration biomarkers with significant findings and limitations. Based upon the available research, we determined that there is currently no validated questionnaire which can be used to assess habitual beverage intake in athletes. Rapid, low resource, and valid methods which can be feasibly utilized in "real world" athletic settings are needed to assess hydration status. Although urine color is inexpensive and can be self-monitored, it is not clear if this method is valid for the assessing hydration status in athletes or the general adult population.

Tools are needed to assess beverage intake and hydration status within the athletic population in order to assist in preventing the adverse effects of dehydration and hypohydration. Understanding the amount of certain beverages consumed (i.e. water, milk, sports drink), especially those aiding in fluid retention, will help athletes and health professionals better meet hydration guidelines and fluid intake recommendations. This dissertation will expand on the importance of monitoring hydration status through proper beverage consumption and urinary indices, within the general adult and collegiate athletic populations. Additionally, one of the following investigations will evaluate the validity and reliability of the BEVQ-15 and urine color. Validation of these tools assesses the agreement of the measurement tool to a reference tool. Reliability of these tools assesses the ability of the tool to provide a similar response for an individual over time. Lastly, this dissertation will evaluate the validation of urine color as a hydration assessment through a novel review of the literature.

REFERENCES

1. Jequier E, Constant F. Water as an essential nutrient: the physiological basis of hydration. *Eur J Clin Nutr.* 2010;64(2):115-123.
2. Casado A, Ramos P, Rodriguez J, Moreno N, Gil P. Types and characteristics of drinking water for hydration in the elderly. *Crit Rev Food Sci Nutr.* 2015;55(12):1633-1641.
3. Sawka MN, Burke, L. M., Eichner, E. R., et al. American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc.* 2007;39(2):377-390.
4. Spano M. KL, Thomas DT. Nutrition for Sport, Exercise and Health. 2018.
5. Sawka MN, Latzka WA, Matott RP, Montain SJ. Hydration effects on temperature regulation. *Int J Sports Med.* 1998;19 Suppl 2:S108-110.
6. Arnaoutis G, Kavouras SA, Angelopoulou A, et al. Fluid balance during training in elite young athletes of different sports. *J Strength Cond Res.* 2015;29(12):3447-3452.
7. Hancock PA, Vasmatzidis I. Effects of heat stress on cognitive performance: the current state of knowledge. *Int J Hyperthermia.* 2003;19(3):355-372.
8. Binkley HM, Beckett J, Casa DJ, Kleiner DM, Plummer PE. National Athletic Trainers' Association Position Statement: Exertional heat illnesses. *J Athl Train.* 2002;37(3):329-343.
9. Castro-Sepulveda M, Cerda-Kohler H, Perez-Luco C, et al. Hydration status after exercise affect resting metabolic rate and heart rate variability. *Nutr Hosp.* 2014;31(3):1273-1277.
10. Casa DJ, Armstrong LE, Hillman SK, et al. National athletic trainers' association position statement: fluid replacement for athletes. *J Athl Train.* 2000;35(2):212-224.

11. Hew-Butler TD, Eskin C, Bickham J, Rusnak M, VanderMeulen M. Dehydration is how you define it: comparison of 318 blood and urine athlete spot checks. *BMJ Open Sport Exerc Med.* 2018;4(1):e000297.

12. Mahan LK RJ. *Krause's food & the nutrition care process.* 14th ed. Louis, MO: Elsevier; 2017.

13. Rhoda KM, Porter MJ, Quintini C. Fluid and electrolyte management: putting a plan in motion. *J Parenter Enteral Nutr*. 2011;35(6):675-685.

14. Popkin BM, D'Anci KE, Rosenberg IH. Water, hydration, and health. *Nutr Rev.* 2010;68(8):439-458.

15. Whitmire SJ. Nutrition-focused evaluation and management of dysnatremias. *Nutr Clin Pract.* 2008;23(2):108-121.

16. Adams JD, Sekiguchi Y, Suh HG, et al. Dehydration Impairs Cycling Performance, Independently of Thirst: A Blinded Study. *Med Sci Sports Exerc.* 2018.

17. Millard-Stafford M, Wendland DM, O'Dea NK, Norman TL. Thirst and hydration status in everyday life. *Nutr Rev.* 2012;70 Suppl 2:S147-151.

18. Strazzullo P, Leclercq C. Sodium. *Adv Nutr.* 2014;5(2):188-190.

19. Ross AC TC, Yaktine AL, et al. *Dietary Reference Intakes: A Risk Assessment Model for Establishing Upper Intake Levels for Nutrients.* Washington, DC: Institute of Medicine (US) Food and Nutrition Board; 1998.

20. Dietary Reference Intake for Water, Potassium, Sodium, Chloride, and Sulfate. *THE NATIONAL ACADEMIES PRESS.* 2004.

21. Drewnowski A, Rehm CD, Constant F. Water and beverage consumption among adults in the United States: cross-sectional study using data from NHANES 2005-2010. *BMC public health.* 2013;13:1068.

22. Maughan RJ, Watson P, Cordery PA, et al. A randomized trial to assess the potential of different beverages to affect hydration status: development of a beverage hydration index. *Am J Clin Nutr.* 2016;103(3):717-723.

23. Clarke MM, Stanhewicz AE, Wolf ST, Cheuvront SN, Kenefick RW, Kenney WL. A randomized trial to assess beverage hydration index in healthy older adults. *Am J Clin Nutr.* 2019;109(6):1640-1647.

24. Desbrow B, Jansen S, Barrett A, Leveritt MD, Irwin C. Comparing the rehydration potential of different milk-based drinks to a carbohydrate-electrolyte beverage. *Appl Physiol Nutr Metab.* 2014;39(12):1366-1372.

25. "athlete". Merriam-Webster.com; 2019.

26. Thomas DT, Erdman KA, Burke LM. American College of Sports Medicine Joint Position Statement: Nutrition and athletic performance. *Med Sci Sports Exerc.* 2016;48(3):543-568.

27. Casa DJ, Guskiewicz KM, Anderson SA, et al. National athletic trainers' association position statement: preventing sudden death in sports. *J Athl Train.* 2012;47(1):96-118.

28. Shirreffs SM, Maughan RJ. Volume repletion after exercise-induced volume depletion in humans: replacement of water and sodium losses. *Am J Physiol.* 1998;274(5 Pt 2):F868-875.

29. Shirreffs SM. The importance of good hydration for work and exercise performance. *Nutr Rev.* 2005;63(6 Pt 2):S14-21.

30. Chang T, Ravi N, Plegue MA, Sonneville KR, Davis MM. Inadequate Hydration, BMI, and Obesity Among US Adults: NHANES 2009-2012. *Ann Fam Med.* 2016;14(4):320-324.

31. Malik VS, Popkin BM, Bray GA, Despres JP, Hu FB. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. *Circulation.* 2010;121(11):1356-1364.

32. The state of obesity: better policies for a healthier America. 2004-2018.

33. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *Jama.* 2014;311(8):806-814.

34. Birch LL. Child feeding practices and the etiology of obesity. *Obesity (Silver Spring, Md).* 2006;14(3):343-344.

35. Lora KR, Hubbs-Tait L, Ferris AM, Wakefield D. African-American and Hispanic children's beverage intake: Differences in associations with desire to drink, fathers' feeding practices, and weight concerns. *Appetite.* 2016;107:558-567.

36. Rosinger AH, K.; Gahche, J.; Park, S. Sugar-sweetened Beverage Consumption Among U.S. Adults, 2011–2014. Vol 270: NCHS Data Brief; 2017.

37. Rosinger A, Herrick, K. Daily Water Intake Among U.S. Men and Women, 2009–2012. Vol 242: National Center for Health; 2016.

38. Hedrick VE, Savla J, Comber DL, et al. Development of a brief questionnaire to assess habitual beverage intake (BEVQ-15): sugar-sweetened beverages and total beverage energy intake. *J Acad Nutr Diet.* 2012;112(6):840-849.

39. Hedrick VE, Comber DL, Estabrooks PA, Savla J, Davy BM. The beverage intake questionnaire: determining initial validity and reliability. *J Am Diet Assoc.* 2010;110(8):1227-1232.

40. Elwan D, Schweinitz P, Wojcicki JM. Beverage consumption in an Alaska Native village: a mixed-methods study of behaviour, attitudes and access. *Int J Circumpolar Health.* 2016;75(1):29905.

41. Hill CE, MacDougall, C. R., Riebl, S. K., et al. Evaluation of the relative validity and test-retest reliability of a 15-Item beverage intake questionnaire in children and adolescents. *J Acad Nutr Diet.* 2017;117(11):1757-1766.e1755.

42. Lora KR, Davy, B., Hedrick, V., et al. Assessing initial validity and reliability of a beverage intake questionnaire in hispanic preschool-aged children. *J Acad Nutr Diet.* 2016;116(12):1951-1960.

43. Yin HS, Johnson M, Mendelsohn AL, Abrams MA, Sanders LM, Dreyer BP. The health literacy of parents in the United States: a nationally representative study. *Pediatrics.* 2009;124 Suppl 3:S289-298.

44. Hooper LP, Myers, E. A., Zoellner, J. M., et al. The impact of health literacy status on the comparative validity and sensitivity of an interactive multimedia beverage intake questionnaire. *Nutrients.* 2016;9(1).

45. Riebl SK, Paone, A. C., Hedrick, V. E., et al. The comparative validity of interactive multimedia questionnaires to paper-administered questionnaires for beverage intake and physical activity: pilot study. *JMIR Res Protoc.* 2013;2(2):e40.

46. Abian-Vicen J, Del Coso J, Gonzalez-Millan C, Salinero JJ, Abian P. Analysis of dehydration and strength in elite badminton players. *PloS one.* 2012;7(5):e37821.

47. How to Maximize Performance Hydration. In: NCAA, ed: Sports, Cardiovascular, and Wellness Nutrition; 2013.

48. Magal M, Cain RJ, Long JC, Thomas KS. Pre-Practice Hydration Status and the Effects of Hydration Regimen on Collegiate Division III Male Athletes. *J Sports Sci Med.* 2015;14(1):23-28.

49. Armstrong LE. Assessing hydration status: the elusive gold standard. *J Am Coll Nutr.* 2007;26(5 Suppl):575S-584S.

50. Convertino VA, Armstrong LE, Coyle EF, et al. American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc.* 1996;28(1):i-vii.

51. Maughan RJ, Shirreffs SM. Rehydration and recovery after exercise. *Sci & Sport.* 2004;19(5):234-238.

52. Maughan RJ, Watson P, Cordery PA, et al. A randomized trial to assess the potential of different beverages to affect hydration status: development of a beverage hydration index. *Am J Clin Nutr.* 2016;103(3):717-723.

53. Vist GE, Maughan RJ. The effect of osmolality and carbohydrate content on the rate of gastric emptying of liquids in man. *J Physiol.* 1995;486 (Pt 2):523-531.

54. Evans GH, Shirreffs SM, Maughan RJ. Postexercise rehydration in man: the effects of osmolality and carbohydrate content of ingested drinks. *Nutrition.* 2009;25(9):905-913.

55. Leiper JB. Intestinal water absorption--implications for the formulation of rehydration solutions. *Int J Sports Med.* 1998;19 Suppl 2:S129-132.

56. Laja Garcia AI, Samaniego Vaesken ML, Partearroyo T, Varela Moreiras G. Validated questionnaire to assess the hydration status in a healthy adult Spanish population: a cross sectional study. *Nutricion hospitalaria.* 2019.

57. Marakis G, Kontopoulou, L., Garofalakis, G., et al. Development and relative validity of a self-administered semi-quantitative drinks frequency questionnaire, validated in a population of university students. *Nutr Diet.* 2019;76(5):532-538.

58. Vanderlee L, Reid JL, White CM, et al. Evaluation of the online Beverage Frequency Questionnaire (BFQ). *Nutrition journal.* 2018;17(1):73.

59. Hedrick VE, Myers EA, Zoellner JM, Duffey KJ, Davy BM. Validation of a rapid method to assess habitual beverage intake patterns. *Nutrients.* 2018;10(1).

60. De Cock N, Van Camp J, Kolsteren P, et al. Development and validation of a quantitative snack and beverage food frequency questionnaire for adolescents. *J Hum Nutr Diet.* 2017;30(2):141-150.

61. Ferreira-Pego C, Nissensohn M, Kavouras SA, et al. Beverage Intake Assessment Questionnaire: Relative Validity and Repeatability in a Spanish Population with Metabolic Syndrome from the PREDIMED-PLUS Study. *Nutrients.* 2016;8(8).

62. Karabudak E, Koksal E. Validity and reliability of beverage intake questionnaire: evaluating hydration status. *Nutr Hosp.* 2016;33(5):577.

63. Koleilat M, Whaley SE. Reliability and Validity of Food Frequency Questions to Assess Beverage and Food Group Intakes among Low-Income 2- to 4-Year-Old Children. *J Acad Nutr Diet.* 2016;116(6):931-939.

64. Muckelbauer R, Libuda L, Kersting M. Relative validity of a self-completion 24 h recall questionnaire to assess beverage consumption among schoolchildren aged 7 to 9 years. *Public Health Nutr.* 2010;13(2):187-195.

65. Marshall TA, Eichenberger Gilmore JM, Broffitt B, Levy SM, Stumbo PJ. Relative validation of a beverage frequency questionnaire in children ages 6 months through 5

years using 3-day food and beverage diaries. *J Am Diet Assoc.* 2003;103(6):714-720; discussion 720.

66. Armstrong LE. Hydration assessment techniques. *Nutr Rev.* 2005;63(6 Pt 2):S40-54.

67. Ersoy N, Ersoy G, Kutlu M. Assessment of hydration status of elite young male soccer players with different methods and new approach method of substitute urine strip. *J Int Soc Sports Nutr.* 2016;13(1):34.

68. Armstrong LE, Maresh CM, Castellani JW, et al. Urinary indices of hydration status. *Int J Sport Nutr.* 1994;4(3):265-279.

69. Kavouras SA, Johnson EC, Bougatsas D, et al. Validation of a urine color scale for assessment of urine osmolality in healthy children. *Eur J Nutr.* 2016;55(3):907-915.

70. Sommerfield LM, McAnulty SR, McBride JM, et al. Validity of Urine Specific Gravity When Compared With Plasma Osmolality as a Measure of Hydration Status in Male and Female NCAA Collegiate Athletes. *Journal of strength and conditioning research.* 2016;30(8):2219-2225.

71. Fernandez-Elias VE, Martinez-Abellan A, Lopez-Gullon JM, et al. Validity of hydration non-invasive indices during the weightcutting and official weigh-in for Olympic combat sports. *PloS one.* 2014;9(4):e95336.

72. Armstrong LE, Soto JA, Hacker FT, Jr., Casa DJ, Kavouras SA, Maresh CM. Urinary indices during dehydration, exercise, and rehydration. *Int J Sport Nutr.* 1998;8(4):345-355.

73. Simerville JA, Maxted WC, Pahira JJ. Urinalysis: a comprehensive review. *Am Fam Physician.* 2005;71(6):1153-1162.

CHAPTER 2: THE FORMULATION AND SENSORY EVALUATION OF A WHEY PERMEATE HYDRATION BEVERAGE

ABSTRACT

Whey permeate, a byproduct of cheese and Greek yogurt manufacturing, boasts a hydration-promoting nutrient profile, suggesting value as an ingredient for functional beverages. However, there are currently no whey permeate-based beverages on the market. Functional ingredients often contribute sensory characteristics that require masking or modification for product success. The objectives of this research were to formulate a whey permeate-based hydration beverage and assess its overall acceptability (9-Point Likert Scale) and sensory attributes (Just About Right Scale (JAR)) for formulation guidance. Healthy adults (n=58; mean age between 31-35 years, 56% female) were recruited from a university campus. Participants provided basic demographic information and completed a questionnaire to assess habitual beverage intake. Participants evaluated four experimental beverage formulas in a randomly assigned order in comparison with a control hydration beverage (lemon-lime Gatorade®). Experimental variables included two flavors (lemon lavender, lemon punch) and sugar content (3%, 5% by weight). Overall, acceptability of experimental beverages was significantly lower to neutral ($\bar{x}$ = 4.5 – 4.9; $p < 0.05$) compared to the control beverage (6.7; “slightly” to “moderately” liked). Just About Right data combined with overall acceptability scores suggested how attributes influenced acceptability. Formulation adjustments are needed before the market potential of a whey permeate-based hydration beverage can be assessed.

INTRODUCTION

Maintaining adequate hydration is critical for optimal bodily functioning.[1] Dehydration is associated with mental fatigue, lack of alertness, and confusion.[2] Though water is essential for replenishing body fluid losses following exercise, the addition of electrolytes, particularly sodium and potassium, can increase the amount of fluid retained.[3] Sports drinks and milk are common fluid replacements since they contain electrolytes (i.e. potassium, sodium, etc.) and carbohydrates such as sucrose and/or lactose.[3,4] Sports drinks and non-fat milk have been recommended as rehydration beverages due to their nutrient profile, which promotes fluid absorption and retention.[5] Carbohydrates in rehydration beverages replenish glycogen stores and can increase fluid retention following exercise.[5-7] The protein in non-fat milk, which is not found in most sports drinks, promotes muscle protein synthesis and may also contribute to rehydration as it decreases the loss of body fluid while maintaining plasma osmolality.[6]

Whey permeate is a powder that is created as a co-product with whey protein concentration and whey protein isolate from the liquid whey byproduct that is expressed during processing of Greek yogurt and many cheeses.[10] Whey permeate is rich in electrolytes, lactose, and whey proteins, including branched-chain amino acids, which may promote hydration.[5] Beverages containing these nutrients, such as non-fat milk, are considered high on the beverage hydration index (BHI).[4] In addition, the micronutrients within whey permeate beverages provide other health benefits which may further enhance the value of this product.[9] However, whey permeate often contributes off-flavor, -odor, and -texture characteristics in food and beverage products, limiting its application. Therefore, the goal of this project was to formulate a whey permeate-based beverage for use in a hydration study. The first objective of this investigation was to formulate whey permeate-based beverages to promote hydration. The second objective was to evaluate the formulated products using validated sensory analysis methods for potential use in a clinical trial.

METHODS

SAMPLE PREPARATION

A base beverage formulation was developed using 84% (by weight) water (Kroger Brand, Cincinnati, OH), 12.6% whey permeate powder (Agri-Mark Inc., Methuen, MA), 3.4% powdered whey concentrate (Heat Stable; (Agri-Mark Inc., Methuen, MA), and 0.15% stabilizer (Ticaloid Pro 192 AGD High Viscosity, TIC GUM, Belcamp, MD). Whey concentrate was used to increase the protein content of the beverage to more closely mimic that of non-fat milk. Ingredients were mixed together, heated to 80ºC to activate the stabilizer with stirring, and then cooled to ambient temperature (21ºC).

Four formulations were designed, targeting sweetness (2 levels of sucrose: 3%, 5% by weight) and flavoring (2 lemon-based flavors) for masking the whey protein and high salt tastes in the whey permeate product. Whey masking agent (0.5% by weight; Gold Coast Ingredients Inc., Commerce, CA) and citric acid (0.33% by weight) were added also to help minimize the salt and whey protein influence on flavor. The two flavors were lemon punch (LP) and lemon lavender (LL) (Gold Coast Ingredients Inc., Commerce, CA), added at 0.5% by weight. These flavors were selected from a variety of flavor options that the research team believed might provide complementary or masking benefit to the whey permeate and those which might be similar to commercially available sports drinks.

Samples for sensory testing included the whey permeate-based beverages (LL3%; LL5%; LP3%; LP5%) and a commercial sports drink (Lemon-Lime Gatorade®, PepsiCo, Purchase, NY). Aliquots of each solution (1 oz.,15 g) were poured into disposable plastic cups (2 oz.; Monogram Company, Columbia, MD) coded with 3-digit beverage identification numbers and sealed with a plastic cap. Samples were stored at a refrigerated temperature (4ºC) until sensory testing.

STUDY PROTOCOL

This investigation consisted of one study session which included completion of a demographic questionnaire, a beverage intake questionnaire, and a sensory panel. The protocol was approved by the Institutional Review Board of Virginia Tech, and all participants were requested to review the written consent form and provide consent prior to enrollment. Individuals aged 18-65 years, with activity levels ranging from sedentary to active, were recruited from a university campus using email listservs and social media advertisements. The population was selected to assess beverage acceptability in a general adult sample. Individuals who were lactose intolerant or allergic to milk proteins were excluded from participation.

The demographic questionnaire captured information on gender, age, ethnicity/race, socioeconomic status, and activity level. Usual beverage intake habits were determined using the 15 category Beverage Intake Questionnaire (BEVQ-15). This validated questionnaire consists of 15 beverage categories and was developed to quantitatively assess habitual beverage consumption over the past 30 days.[7]

SENSORY PROTOCOLS

Participants completed testing at the Virginia Tech Sensory Evaluation Laboratory in the Department of Food Science and Technology. Participants were seated in individual sensory evaluation booths; each booth was equipped with a sliding hatch to receive and return beverage samples. Participants were given printed forms (scorecards: acceptability, JAR) to complete for each sample (n=5) they tasted. Product acceptability was assessed using a 9-point Likert-type scale (1=dislike extremely; 5= neither like nor dislike; 9=like extremely) as well as the Just About Right (JAR) scale[8] and given to participants in a randomly assigned order. The JAR scale

(1 = “too thin/too light/too little”, 3= “just about right”, 5 = “too thick/too dark/too much”) was used to evaluate levels of a specific attribute of the product against participant’s self-perception of the ideal.[8] Attributes evaluated on the JAR scale included appearance, smell, taste, mouthfeel, and aftertaste.

MINERAL ANALYSIS

Sodium, magnesium, inorganic phosphorus, sulfur, chlorine, potassium, and calcium concentrations of each whey permeate-based beverage, as well as the commercial sports drink, were measured by emission spectroscopy using Inductively Coupled Plasma (ICP) technique (Thermo Electronic Corporation, X-Series ICP-MS, Waltham, MA).[9] Each whey permeate-based beverage and the commercial sports drink were centrifuged at 18500 ×g for 15 minutes to reduce viscosity and remove debris. Sample digestion was modified from the previous studies by diluting each sample at 1:10 (v/v) with 4% nitric acid and mixed well by vortex in order to decompose the sample for the release of minerals and accurate analysis.[9,10]

STATISTICAL ANALYSES

Descriptive statistics were used to characterize study participant demographics and to describe acceptability data (mean ± SD). Sample size was not estimated prior to this investigation; however, our sample size is consistent with previous literature is consistent with that used in existing food sensory research studies. One participant was excluded from analysis due to incomplete demographic questionnaire data and JAR data. Independent sample t-test was used to evaluate gender differences. Paired, two-sample t-tests (one-tail) were completed between each formulation and each formulation to the commercial control beverage; alpha was preset at 5%. Independent sample t-test was used to evaluate differences in major electrolyte composition. Statistical analyses were performed using Microsoft Excel 2010 and IBM SPSS Statistics statistical analysis software (Version 24). Analysis of JAR data, in combination with acceptability scores for overall acceptability, was completed using XL-STAT Sensory (Microsoft Excel).[11] Penalty analysis and mean drop were calculated to determine guidance for potential improvements that might positively affect acceptability scores.

RESULTS

Participants' ages ranged from 18-65 years of age (n=58; 55.2% female) (Table 2.1). The majority (93%) of the participants considered themselves healthy (subjective yes/no response), and most (79%) reported that they were moderately active and incorporate hydration strategies into their daily life. Daily hydration strategies included selecting water (44%), milk or other dairy products (28%), sports drinks (12%), energy drinks (4%), pop/soda (7%), and/or other beverages (12%). "Other" beverages included: tea, coffee, lemonade, and seltzer water.

Table 2.1. Participant Characteristics

Participant Characteristics	**Value (n,%)**
Sex	
Male	25 (43.1)
Female	32 (55.2)
Age Range, yrs	
18-30	33 (56.9)
31-50	13 (22.4)
51+	11 (19.0)
Ethnicity	
American Indian/Alaskan Native	2 (3.4)
Black/African American	3 (5.2)
White	36 (62.1)
Asian	15 (25.9)
Other Race	1 (1.7)
Activity Level	
Sedentary or Inactive	0 (0)
Recreationally Active	19 (32.8)
Moderately Active	33 (56.9)
Extremely Active	5 (8.6)

Five participants did not provide complete data for the BEVQ-15 and thus were excluded from BEVQ analysis (n=53). Mean total daily beverage intake was 62.3 ± 32.2 fl oz. and 328 ±

586 kcal/day (from beverages). Most participants reported consuming water (81%) and milk (89%) at some level. Mean water and milk intake were 33.2 ± 18.3 fl oz. and 5.6 ± 7.4 fl oz., respectively. Female participants consumed 34.27 ± 19.07 fl oz. water and 5.75 ± 8.68 fl oz. milk per day, and male participants consumed 31.8 ± 17.55 fl oz. water and 5.55 ± 5.72 fl oz. milk per day with no significant differences by gender ($p > 0.05$). However, male participants consumed significantly ($p < 0.005$) more energy/sports drinks than the female participants (males 1.58 ± 3.72 fl oz.; females 0.30 ± 0.77 fl oz.). Only 16% of participants reported consuming these beverages, thus mean consumption levels were low for this beverage category.

Acceptability of the four formulated whey permeate beverages approached the neutral score (5=neither like nor dislike) (Figure 1). The commercial sports drink (control) had a mean of 6.7, corresponding to “slightly” to “moderately” accepted. There was a significant difference ($p < 0.0001$) between each test beverage compared to the commercial beverage but no differences among mean acceptability scores for experimental beverages. For the tested population, responses did not follow a normal distribution and histograms illustrate the distribution was typically bi-modal for the experimental beverages. The experimental LL formulation with 5% sucrose (LL5%) had 58% of respondents selecting ‘neutral’ or higher on the acceptability scale.

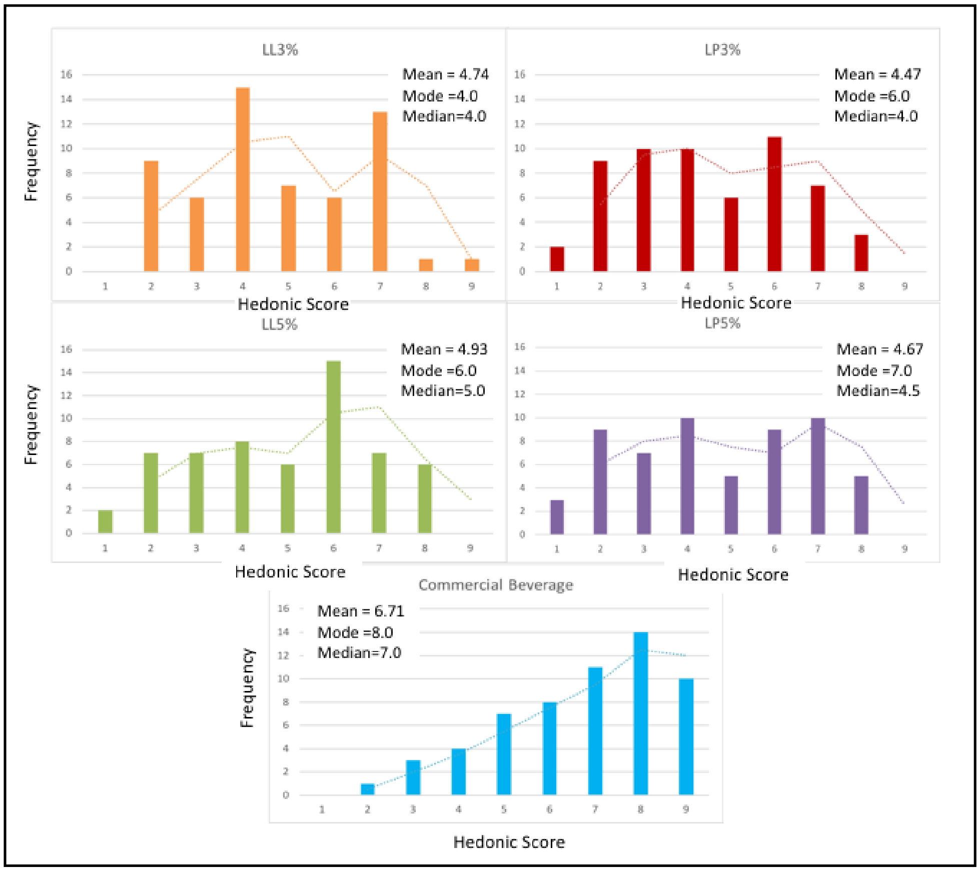


Figure 2.1 *Histograms of acceptability ratings (Mean ± SD; n=58; 1 = Dislike Extremely, 5=neither like nor dislike, 9= Like Extremely) of experimental and control beverages. LL = Lemon Lavender; LP = Lemon Punch; 3%, 5%: sucrose by weight; Commercial sports drink (Lemon-Lime Gatorade, PepsiCo™, Chicago, IL).*

Just About Right (JAR) scores were used to further clarify how respondents compared the prototype formulations to their 'ideal' hydration beverage concept (Figure 2). For each attribute, we identified significant differences ($p<0.05$) in JAR values between the means of each experimental formulation and the commercial beverage. No beverage, including the commercial beverage, received the ideal JAR score for all attributes.

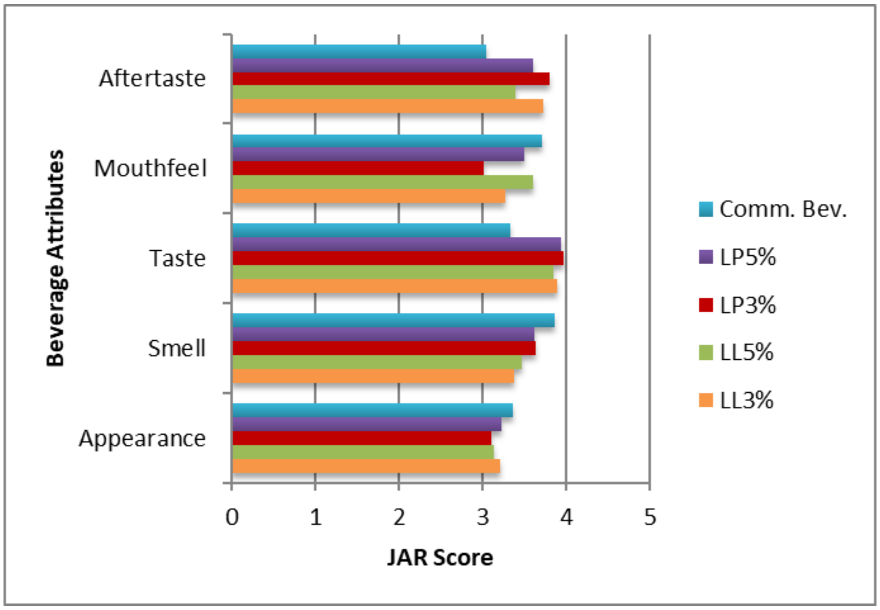


Figure 2.2. *Just About Right (JAR) (Mean ± SD; n=58) ratings for experimental and commercial beverages. LL = Lemon Lavender; LP = Lemon Punch; 3%, 5%: sucrose by weight; Commercial sports drink (Lemon-Lime Gatorade, PepsiCo™, Chicago, IL). JAR scale for attributes included Appearance: 1=too light, 5=too dark; Mouthfeel: 1=too thin, 5=too thick; Smell, Taste, Aftertaste: 1=too little, 5=too much; 3=Just about Right for all attributes.*

The distribution of JAR responses for each attribute within each formulation helps assess the impact of the non-JAR ratings (JAR=3.0 value) on overall acceptability of the product. A large change in mean value (mean drop) and a large percentage of the population indicates a potential for formulation modification to improve acceptability (Figure 3). The commercial beverage and two experimental formulations (LP3%, LL5%) had no attributes that significantly influenced the overall mean acceptability score. Significant differences (p<0.05) between the too light/too dark (non-JAR) proportions of the population were noted for appearance (LL3%, LP5%) with the 'too dark' response creating a positive influence (>1.3) on acceptability score. Mouthfeel (too thick) was detrimental to acceptability score for LL3% (p<0.05). In Figure 3, note that mouthfeel (in the lower right quadrant LL3%) depresses the acceptability score for a large proportion (33%) of the population. Mean drop values near 0 (-1 to 1) and/or with small proportion of the population (<20%) indicate small penalties and are not actionable (LL5%).

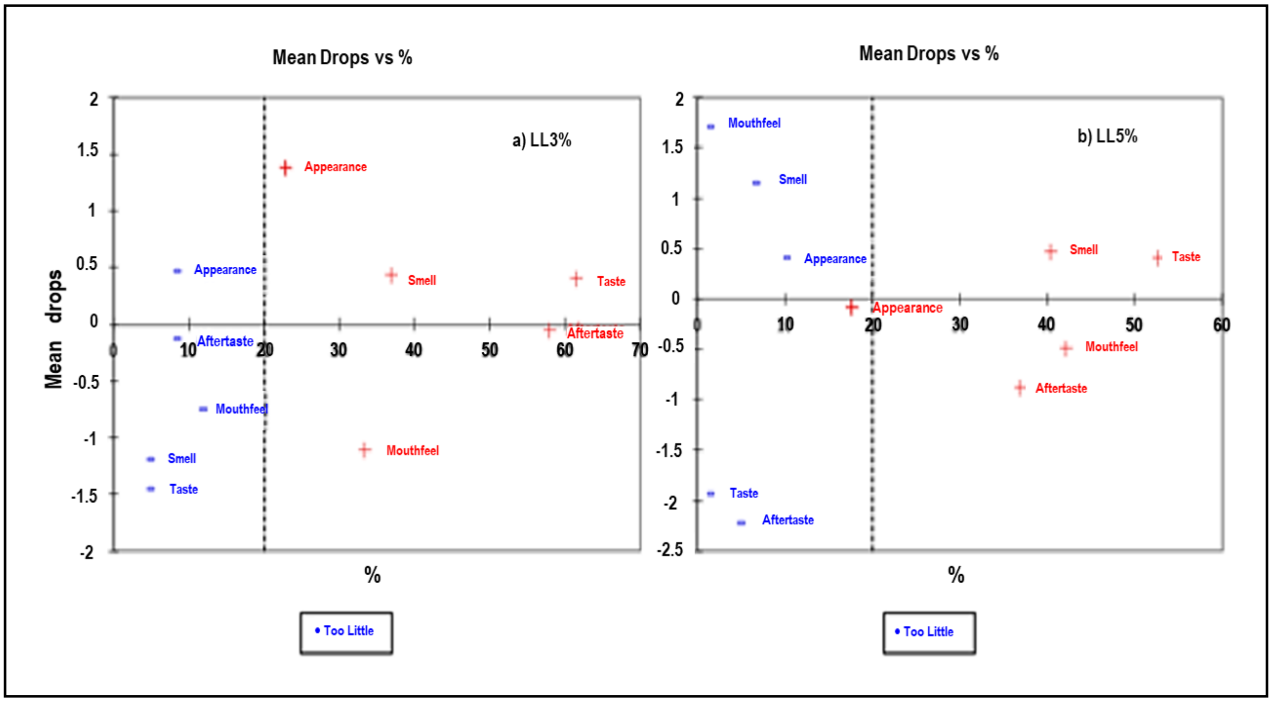


Figure 2.3. *Illustration of penalty analysis and mean drop for lemon lavender (LL) whey-based hydration beverage at two sucrose levels (3% sucrose, 5% sucrose).*

The formulated beverage delivered a combination of nutrients valuable for hydration, including whey proteins, carbohydrates (lactose, sucrose), minerals, and electrolytes common in milk (Table 2.2). For major electrolytes (sodium, magnesium, inorganic phosphorus, potassium, and calcium), the whey permeate beverage was found to have significantly higher ($p \leq 0.05$) electrolyte composition than the commercially available hydration beverage.

Table 2.2. Comparison of hydration components in nonfat milk (USDA ARS 2017) and experimental hydration beverage formula.

Component	Whey Permeate Beverage (Per 8 Fl Oz, 238.72 g)	Nonfat Milk (Per 8 Fl Oz, 244.8 g)
ENERGY	133.52 kcal	83.00 kcal
WATER	198.95 g	222.38 g
PROTEIN	7.81 g	8.25 g
LIPID (TOTAL)	0.40 g	0.20 g
CARBOHYDRATE (BY DIFFERENCE)*	25.48 g	12.14 g
CALCIUM	181 mg	299 mg
MAGNESIUM	40 mg	27 mg
PHOSPHORUS	296 mg	247 mg
POTASSIUM	693 mg	382 mg
SODIUM	211 mg	103 mg

***** By difference is included to address the sum of the nutritionally available carbohydrate from the total carbohydrate. For the whey permeate beverages, this was based upon the 3% sucrose formulation

DISCUSSION

To our knowledge, this is the first investigation to formulate a whey permeate-based beverage for the general adult population with the intention to promote hydration, following exercise. The beverage formulations did not meet the targeted mean acceptability score goal of 6 or higher. Typically, product developers strive for a mean acceptability score close to the market leader, which typically is at or above the 'like moderately' category (mean of 7 or higher on a 9-point scale) to suggest a potential for commercial success;[17] this suggests that additional formulation modifications are needed to improve acceptability for potential commercial success. For example, flavors that are compatible with dairy products, such as vanilla, chocolate or caramel could be utilized. In addition, slightly decreasing the amount of electrolytes, similar to that of non-fat milk could improve acceptability. If targeted for a clinical trial, formulation improvement would promote participant compliance. JAR data provides some guidance for improving the formulation. It is difficult to interpret the exact influence on JAR responses which may be attributed to flavors in the whey permeate or concentrate, amount of flavoring added, the lavender or punch influence on the flavoring, or the combination. In general, of the whey permeate formulations tested, the LL5% had the best potential for further development; this formulation presented the greatest acceptability of the whey-permeate based beverages.

In order to efficiently retain and replenish the fluids lost through exercise, beverages that contain electrolytes, such as sports drinks, dairy products, and specific foods are recommended.[12-14] The inclusion of electrolytes in this study's whey permeate beverage would likely classify this beverage's BHI similar to that of skimmed milk.[4] This whey permeate beverage contains greater amounts of carbohydrates and electrolytes than non-fat milk. In comparison to most sports drinks, the whey permeate beverage contains branched-chain amino acids necessary for muscle rebuilding and thought to promote hydration by maintaining plasma osmolality.[6] Therefore, this formulation may be useful for increasing hydration status more than the current rehydration beverages that are on the market. Specifically, sodium intake increases plasma volume, promotes thirst, and increases the absorption of glucose in the small intestine.[14,15] However, due to the high content of salt in whey permeate, Beucler et al.[18] found that lower concentrations of the by-product, incorporated into a commercial beverage for their experimental study, were more acceptable.[16] Together with our findings, this suggests that the amount of whey permeate and whey concentrate should be reduced (i.e. 25% of beverage volume) or masked with a higher concentration of sugar, flavoring, or an effective masking ingredient. In addition, reducing the electrolyte composition of the whey-permeate beverage, similar to the non-fat milk formulation, could aid in improving acceptability.

This study had several strengths. First, the use of whey permeate as a hydration beverage ingredient is novel due to the use of an undervalued by-product of dairy processing. Second, the study population consisted of individuals who were aware of the importance of consuming beverages for hydration purposes and who regularly consumed water and milk making them ideal participants for testing the whey-based formulas. The study utilized validated food sensory evaluation methodologies commonly used in commercial development of food products. We acknowledge the limitation of a small sample size (n=58) that did not represent the general population at large.

Future efforts should evaluate further modifications to the formulation before market potential can be assessed. The flavor selection could better complement the beverage as a dairy product, such as vanilla or caramel to improve coloration (appearance). Particular attention should be focused upon factors that affect mouthfeel, especially as integrated with taste and aftertaste. This could be accomplished by reducing the concentration of whey permeate or concentrate to slightly reduce the mineral, protein, and lactose (carbohydrate) composition, as it may have been too dense.

REFERENCES

1. Jequier E, Constant F. Water as an essential nutrient: the physiological basis of hydration. *Eur J Clin Nutr.* 2010;64(2):115-123.
2. Benton D, Young HA. Do small differences in hydration status affect mood and mental performance? *Nutr Rev.* 2015;73 Suppl 2:83-96.
3. Maughan RJ, Shirreffs SM. Rehydration and recovery after exercise. *Sci & Sport.* 2004;19(5):234-238.
4. Maughan RJ, Watson P, Cordery PA, et al. A randomized trial to assess the potential of different beverages to affect hydration status: development of a beverage hydration index. *Am J Clin Nutr.* 2016;103(3):717-723.
5. Desbrow B, Jansen S, Barrett A, Leveritt MD, Irwin C. Comparing the rehydration potential of different milk-based drinks to a carbohydrate-electrolyte beverage. *Appl Physiol Nutr Metab.* 2014;39(12):1366-1372.
6. Ishihara K, Kato Y, Usami A, et al. Electrolyte-free milk protein solution influences sodium and fluid retention in rats. *J Nutr Sci.* 2013;2:e8.
7. Hedrick VE, Savla J, Comber DL, et al. Development of a brief questionnaire to assess habitual beverage intake (BEVQ-15): sugar-sweetened beverages and total beverage energy intake. *J Acad Nutr Diet.* 2012;112(6):840-849.
8. Meilgaard MC, Civille, G.V., Carr, B.T. *Sensory Evaluation Techniques.* 5th ed. New York: CRC Press, Taylor & Francis Group; 2016.
9. Watanabe M, Asatsuma M, Ikui A, et al. Measurements of several metallic elements and matrix metalloproteinases (MMPs) in saliva from patients with taste disorder. *Chemical senses.* 2005;30(2):121-125.

10. Menegario AA, Packer AP, Gine MF. Determination of Ba, Cd, Cu, Pb and Zn in saliva by isotope dilution direct injection inductively coupled plasma mass spectrometry. *The Analyst.* 2001;126(8):1363-1366.

11. Lawless HT, and Heymann, H. . *Sensory Evaluation of Food, Principles and Practices.* 2nd ed: Springer Science+Business Media, LLC, 2010.

12. Dairy Permeate Standard. *American Dairy Products Institute.* 2014.

13. Smith ST, Metzger L, Drake MA. Evaluation of whey, milk, and delactosed permeates as salt substitutes. *J Dairy Sci.* 2016;99(11):8687-8698.

14. Cássia Pegoretti AECA, Fúlvia de Barros Manchado-Gobatto, Caroline Dario Capitani. Milk: An Alternative Beverage for Hydration? *FNS.* 2015;6:547-554.

15. Peacock OJ, Thompson D, Stokes KA. Voluntary drinking behaviour, fluid balance and psychological affect when ingesting water or a carbohydrate-electrolyte solution during exercise. *Appetite.* 2012;58(1):56-63.

16. Beucler J, Drake M, Foegeding EA. Design of a Beverage from Whey Permeate. *J Food Sci.* 2005;70(4):S277-S285.

CHAPTER 3: EVALUATION OF METHODS TO RAPIDLY ASSESS BEVERAGE INTAKE AND HYDRATION STATUS IN COLLEGIATE ATHLETES

ABSTRACT

Maintaining proper hydration is crucial for all individuals but especially for athletes, who experience quicker fluid turnover rates. Beverage consumption recommendations have been established for the general population and the athletic population in order to promote adequate hydration. The Beverage Intake Questionnaire (BEVQ-15) is a quick and reliable food frequency questionnaire that quantifies habitual beverage intake, which has been validated in children and adults. However, it has not been evaluated in the athletic population and there is no beverage consumption questionnaire that has been validated in collegiate athletes. The purpose of this investigation was to evaluate the validity and reliability of the BEVQ-15 in collegiate athletes. Additionally, this investigation evaluated urine color (UC) as a hydration biomarker compared to urine specific gravity (USG), in collegiate athletes. NCAA Division 1 collegiate athletes (n=120; 54% female; age 19±1yrs) from Southwest Virginia were recruited. The study consisted of three sessions on nonconsecutive days, with one day being a weekend day. The participants completed a 24-hr recall at each study session. At the first and third sessions, each participant completed the BEVQ-15 and provided a urine sample to determine UC and USG. Total fluid intake (fl oz) in the athletes was 111±106.9 and 108±42 using the BEVQ-15 and multiple 24-hr dietary recalls, respectively, which was not significantly different. which was not statistically different. There was a significant correlation ($p \leq 0.05$) between the BEVQ-15 and dietary recall data for total beverage intake for fl oz and kcal (r =0.413 and r=0.465, respectively). Mean urine color rating was UC_{sub} 3.4±1.5 and UC_{res} 3.5±1.7. There was also a strong correlation between UC_{sub} and UC_{res} and USG measures (r=0.67 and r=0.884; $p \leq 0.05$, respectively). Therefore, these

assessment tools demonstrated reasonable validity, and can be used as practical methods to determine if athletes are meeting their hydration needs.

Keywords: beverage consumption, hydration status, validity, reproducibility, collegiate athlete

INTRODUCTION

Adequate hydration is essential for the human body to properly function. Water can be lost through various mechanisms including the renal system, gastrointestinal absorption, the respiratory system and sweat loss.[1,2] As fluids are lost, fluid consumption must increase to ensure that the body maintains euhydration. Euhydration occurs when the body's water content is balanced.[3] However, when body water losses exceed 1-2%, dehydration will result.[1] Moreover, when body water losses exceed 2-4%, hypohydration results.[1] The loss in body water can lead to adverse health effects, such as cognitive impairment, fatigue, increased cardiovascular strain, and increased resting metabolic rate.[4-7] Therefore, replenishing the fluids lost with certain beverages can help to the prevent these adverse effects.

Fluid consumption recommendations are slightly different for the general population and the athletic population. The National Academy of Medicine established the adequate intake of plain water for males and females, 3.7 L/day and 2.7 L/day, respectively.[8] Research has shown that consuming beverages other than water can further enhance fluid retention.[3,10-14] This is especially true when there is excessive sweat loss, due to exercise. Consuming beverages that are high in electrolytes (i.e. sodium and potassium) and specific macronutrients may increase fluid retention, and promote rapid rehydration.[10,14] Recommendations for athletic populations are different before, during and after exercise and should be individualized to optimize performance.[3,12] Assessing usual beverage intake is challenging. The BEVQ-15 is a rapid, quantitative food frequency questionnaire that was developed to measure habitual beverage consumption in adults.[15] This questionnaire consists of fifteen beverage categories and an "other" category that is available for the beverages that are not listed. This 15-item questionnaire has been validated in several populations, including children and adolescents.[16-23] However, there is currently no validated, practical method to measure beverage consumption habits in athletes.

Hydration status can also be assessed using urinary and blood/plasma indices. These include urine osmolality, urinary specific gravity (USG), urine volume, urine color (UC), and plasma/serum osmolality.[3,14] Serum osmolality is considered the gold standard of assessing hydration status; however, this method is invasive and expensive.[24] Practical indicators of hydration status, such as UC and urine volume, are more cost-effective and time efficient for a real-world setting.[3] Armstrong et al., developed and eight-scale UC chart for assessing hydration status on a daily basis.[25] The colors range from very pale yellow to dark brown-green and the colors correspond with hydration. Lighter colors indicate euhydration, while darker colors indicate mild to severe dehydration.[25] However, there is still uncertainty on the validity of UC as a hydration indicator within the athletic population. Therefore, the two study objectives were as follows. The first objective was to evaluate the validity and reliability of the BEVQ-15 for collegiate athletes, compared to three 24-hr recalls. The second objective was to evaluate UC as a hydration biomarker, compared to USG, in collegiate athletes.

METHODS

PARTICIPANTS

Participants were recruited via email, flyers, and individual team meetings with NCAA Collegiate athletes from Virginia Tech and Radford University between April 2017-August 2019. Sports included football, basketball, volleyball, soccer, baseball, softball, tennis, golf, dance, cross country, track & field, and swimming. Eligibility criteria included being a healthy (i.e. uninjured or not demonstrating an acute illness that would preclude from participation in competition) NCAA Division 1 collegiate athlete at either university, who was aged 18 years or older. The athletes that met these criteria and demonstrated interest in participating after a recruitment meeting with the team were individually contacted via email or text messaging to schedule the first session. At the first session, the participants provided written informed consent. This research was approved by the Virginia Tech Institutional Review board.

EXPERIMENTAL PROCEDURE

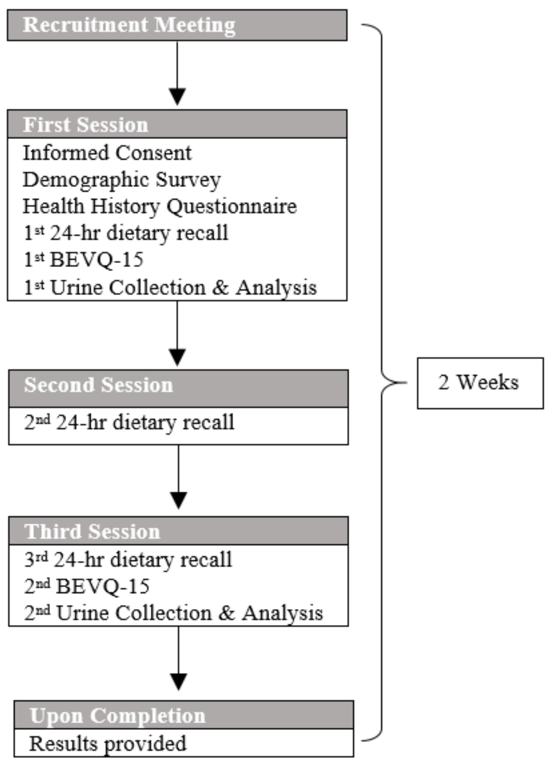


Figure 3.1 Study Protocol.

Each participant completed three study sessions, on nonconsecutive days, which were completed within a two-week period (Figure 3.1). Sessions took place 30-40 minutes prior to team practice or weight lifting sessions. The time of the practice sessions were determined by the teams' coaching staff. For the first session, each participant signed the written informed consent form and completed a demographic survey and health history questionnaire. The survey asked the participant to provide their age, sex, sport, year of eligibility, race/ethnicity, and role on the team. The questionnaire asked the participant whether they had any medical conditions and if they were currently taking medications or supplements. The height and weight was then measured in order to calculate body mass index (BMI). Weight was measured using a Tanita Bf-350 Scale while height was determined from the official athletic rosters based on the most recent physical examination.

Next, each participant completed the BEVQ-15 for the first time (see Appendix A).[17] If a participant was consuming a beverage that was not listed, they were instructed to write it in the "other" category. Following the completion of the BEVQ-15, a trained research assistant administered a face-to-face 24-hr dietary recall of the previous day, using the Automated Multiple Pass Method.[26,27] Food diagrams were provided to each participant in order to obtain the appropriate portion sizes. Next, the participant was asked to provide a mid-stream urine sample, 30 minutes prior to practice. Each participant was provided with the eight-scale UC chart and a white background in order to evaluate their UC (see Figure 2), which was blinded to the researcher.[25] The researcher then evaluated the UC using the same color chart and recorded the results. Lastly, the researcher used a hand-held refractometer (Atago, 4410 PAL-10S, Tokyo, Japan) to determine the USG, in duplicates. The refractometer was cleared using 2-3 teaspoons of deionized water.

The second session consisted only of a second 24-hr recall, which was scheduled at a convenient time and location for the participant. The third session included the completion of the third 24-hr recall, the second BEVQ-15 and providing the second urine sample for analysis of UC and USG.

The three 24-hr dietary recalls were analyzed using Nutrition Data Systems of Research (NDSR), version 2013 (University of Minnesota, Minneapolis, MN). The BEVQ-15 was analyzed using a formulated scoring template, which determined the amount of fluid ounces and calories of each beverage category consumed in a day. The UC chart (see Figure 3.2) consists of 8 different colors that range from very pale yellow to brown-green.[25] Colors 1-2 indicated well-hydrated, 3-6 indicated mild dehydration, and $\geq$ 7 indicated severe dehydration.[25] Additionally, UC on the urine color chart corresponds with specific USG values.[25,28] For instance, UC 1-2 corresponds with USG <1.010; UC 3-4 corresponds with 1.010-1.020; UC 5-6 corresponds with USG 1.021-1.030; UC 7-8 corresponds with USG >1.030.[28]

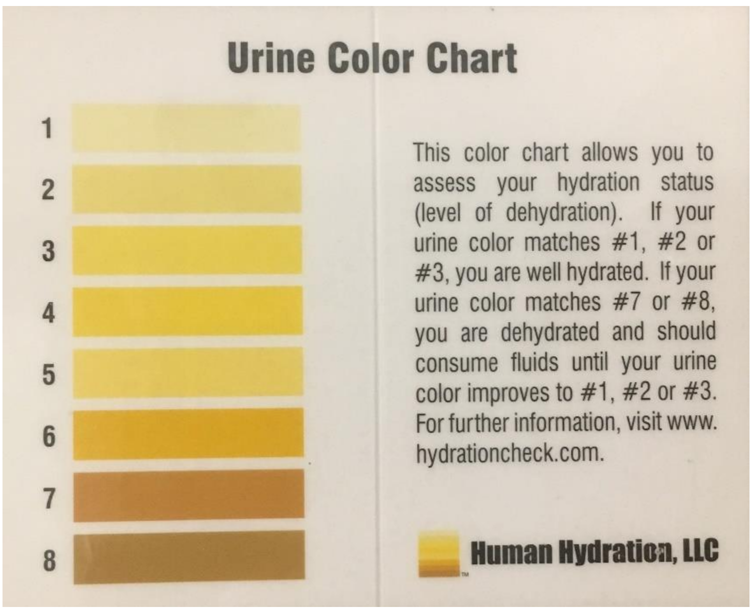


Figure 3.2 Urine Color Chart.[25]

STATISTICAL ANALYSIS

Descriptive statistics were used to examine participant characteristics (mean ± SEM; frequency). Statistical analyses were performed using IBM SPSS Statistics 25 and statistical significance was set to $p \leq 0.05$.

Beverage Consumption

The reference measure used to assess BEVQ validity was beverage intake determined using multiple 24-hour recalls.[29] Spearman's rank-order correlation was used to determine the correlation between the second administration of the BEVQ-15 and the 3-day averaged 24-hr dietary recalls. The second BEVQ was utilized since it corresponded most closely with the timeframe of dietary recall collection. In addition, the level of agreement between two dietary assessment methods can be determined through the measurement error.[29] Paired T-test and Bland Altman plots were used to determine the agreement between the BEVQ-15 and the 24-hr dietary recalls. Reliability was assessed using Pearson's correlational analyses of the BEVQ-15 at two time-points. Correlation coefficients between weak (r=0.3-0.49); moderate (r=0.50-0.69); or strong (r=0.70-1.00).[30]

Urinalysis

Spearman's correlation was used to determine associations between USG and UC (subject and researcher); the average of the duplicate USG measures was used in analysis. Reliability was assessed using a Pearson's correlation analyses of UC, between subject and researcher assessment, at both time points. A Fisher's r-to-z transformation compared the correlation coefficients for UC ratings between subject and researcher assessments. Cohen's kappa statistic was used to determine associations between USG and UC (researcher and subject) and inter-rater reliability between researcher and subject's assessment of UC. Spearman's correlation was used to determine the relationship between USG and total beverage intake (BEVQ-15) at each time point. BEVQ-15 data was also examined for potential outliers.

RESULTS

PARTICIPANT CHARACTERISTICS

One hundred and twenty NCAA Division 1 collegiate athletes completed the study (0% attrition). The participant characteristics are presented in Table 3.1. Participants were balanced across sexes, and mostly of normal weight status, according to BMI (79%). Over half of the sample population was within their freshman and sophomore year of eligibility (55%) and most participants were soccer, cross country, basketball, and swimming athletes (62.5%).

Table 3.1 Participant Characteristics.

Participant Characteristics	Total	Male	Female
Number of participants, n(%)	120	55 (45.8)	65 (54.2)
University		1.45 ± 0.07	1.51 ± 0.06
1=Virginia Tech	62 (51.7)	30 (54.5)	32 (49.2)
2=Radford University	58 (48.3)	25 (45.5)	33 (50.8)
Age (y)	19.8 ± 0.13	20.11 ± 0.19	19.49 ± 0.17
18, n(%)	27 (22.5)	7 (12.7)	20 (30.8)
19, n(%)	29 (24.2)	12 (21.8)	17 (26.2)
20, n(%)	28 (23.3)	16 (29.1)	12 (18.5)
21, n(%)	22 (18.3)	13 (23.6)	9 (13.8)
22, n(%)	8 (6.7)	2 (3.6)	6 (9.2)
23, n(%)	6 (5.0)	5 (9.1)	1 (1.5)
BMI (kg/m^2)	23.2 ± 0.24	23.8 ± 0.30	22.8 ± 0.37
BMI Categories:			
Underweight, n(%)	0 (0)	0 (0)	0 (0)
Normal Weight, n(%)	95 (79.2)	39 (32.5)	56 (46.7)
Overweight, n(%)	24 (20.0)	16 (13.3)	8 (6.7)
Obese, n(%)	1 (0.8)	0 (0)	1 (0.8)
Year of Eligibility	2.4 ± 0.11	2.45 ± 0.15	2.38 ± 0.16
1=Freshman, n(%)	35 (29.2)	13 (23.6)	22 (33.8)
2=Sophomore, n(%)	31 (25.8)	16 (29.1)	15 (23.1)
3=Junior, n(%)	28 (23.3)	15 (27.3)	13 (20.0)
4=Senior, n(%)	21 (17.5)	10 (18.2)	11 (16.9)
5=Graduate Student, n(%)	5 (4.2)	1 (1.8)	4 (6.2)
Sport			
Tennis	11 (9.2)	6 (10.9)	5 (7.7)
Cross Country	17 (14.2)	7 (12.7)	10 (15.4)
Swimming	15 (12.5)	5 (9.1)	10 (15.4)
Baseball	6 (5.0)	6 (10.9)	0 (0.0)
Volleyball	15 (12.5)	0 (0.0)	15 (23.1)
Basketball	16 (13.3)	16 (29.1)	0 (0.0)
Dance	1 (0.8)	0 (0.0)	1 (1.5)
Soccer	27 (22.5)	10 (18.2)	17 (26.2)
Track & Field	2 (1.7)	1 (1.8)	1 (1.5)
Football	1 (0.8)	1 (1.8)	0 (0.0)
Softball	5 (4.2)	0 (0.0)	5 (7.7)
Golf	4 (3.3)	3 (5.5)	1 (1.5)

All values are express in Mean ± SEM

BEVERAGE CONSUMPTION

Validity

BEVQ-15 validity results are presented in Table 3.2. Total beverage intake from each administration of the BEVQ-15 ranged from 20.29 to 538.57 (fl oz) and 22.86 to 1157 (fl oz), for BEVQ and 1 and 2, respectively. These values appeared plausible based upon the sizes of the athletes and intensity of sport; therefore, all available data was used for analysis. Water presented minimal differences and moderate to strong significant correlations when assessing validity ($r=0.459$, $p\leq0.05$). Total beverage intake also demonstrated moderate validity for fl oz and kcal ($r=0.413$-0.465, $p\leq0.05$, respectively). Of the 15 beverage categories, including total beverage intake, five beverage categories did not demonstrate validity: diet or artificially sweetened soft drink (fl oz and kcal), coffee/tea – sweetened/milk/cream (fl oz and kcal), hard liquor (fl oz and kcal), and coffee/tea – black (kcal). All other categories, including total beverage intake were significantly correlated for fl oz and kcal ($r=0.297$-0.686, $p\leq0.05$) (See Table 3.2). Bland-Altman plots were utilized to assess the level of agreement between methods for major beverage categories. The mean differences for total beverage intake were 3.74 fl oz (CI: -14, 21) and 141 kcal (CI: 70, 212), respectively. The Bland-Altman plots (Figure 3.3) demonstrated a strong agreement (98% and 94%, respectively) between the two methods.

Reliability

Reliability results of the BEVQ-15 are presented in Table 3.3. With regard to test-retest reliability, insignificant correlations were found between only two beverage categories, nut milk (kcal) and coffee/tea – black (kcal). All other beverage categories, including total beverage intake, demonstrated reliability ($r=0.294$-0.842, $p\leq0.05$) (see Table 3.3). Water, energy/sports drinks, and total beverage intake presented moderate to strong significant correlations ($r=0.69$-0.79, $p\leq0.05$).

Table 3.2 Evaluation of Validity of BEVQ-15

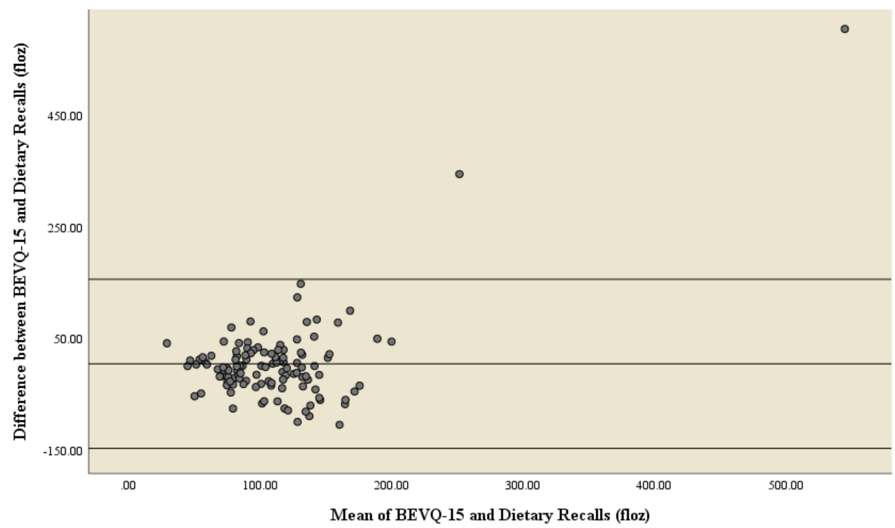
Beverage Category	Validity[a] BEVQ-15[a]	24-h Recalls	Difference[b]	Correlation (r)
	←Mean±SD→		Mean±SEM	
Water (fl oz)	77.7±104.6	82.1±39.1	-4.4±9.0	0.459*
100% Fruit Juice				
fl oz	2.3±5.8	1.2±3.1	1.1±0.6	0.297*
Kcal	40±103	22±55	19±10	0.297*
Sweetened Fruit Juice				
fl oz	1.6±5.5	1.3±3.3	0.3±0.5	0.335*
Kcal	23±78	19±48	-4±7	0.335*
Whole Milk, Reduced Fat Milk, or Chocolate Milk				
fl oz	4.6±8.5	3.5±4.7	1.1±0.7	0.405*
Kcal	91±168	69±94	21±15	0.419*
Low Fat or Fat Free Milk, Buttermilk, or Soy Milk				
fl oz	1.9±4.7	1.2±3.9	0.6±0.3*	0.432*
Kcal	23±57	15±47	8±4*	0.430*
Nut Milk				
fl oz	1.0±2.7	0.5±1.3	0.4±0.2*	0.686*
Kcal	1±7	5±13	-4±1*	0.333*
Regular Soft Drink				
fl oz	0.9±1.8	1.3±4.9	-0.4±0.4	0.384*
Kcal	11±24	17±65	-5±6	0.385*
Energy & Sports Drink				
fl oz	11.0±17.2	9.5±13.8	1.5±1.3	0.672*
Kcal	154±241	133±193	21±18	0.670*
Diet or Artificially Sweetened Soft Drink (fl oz)	0.4±1.7	0±2	0±0.2	0.062
Sweet Tea				
fl oz	0.8±2.6	1± 4	0±0.3	0.479*
Kcal	8±26	8±37	0±3.2	0.475*
Coffee/Tea – Black				
fl oz	2±4.7	4±6	-2.3±0.5*	0.351*
Kcal	1±5.5	2±2	-1±1	0.088
Coffee/Tea – Sweetened/Milk/Cream				
fl oz	3.2±5.2	0±0	3.2±0.5*	0.113
Kcal	16±32	0±2	-15±3*	0.166
Wine				
fl oz	0.3±1.1	0±1	0±0.1	0.497*
Kcal	6±22	6±28	0±2.3	0.497*
Hard Liquor				
fl oz	0.2±0.6	0±0	0.2±0.1*	-0.043
Kcal	14±40	1±8	13±4*	-0.043
Beer				
fl oz	1.0±2.7	2±7	-0.6±0.6	0.400*
Kcal	10±28	16±77	-6±6	0.402*
Total Sugar-Sweetened Beverages				
fl oz	16.4±21.6	13±16	3.0±1.9	0.480*
Kcal	211±293	178±214	33±25	0.508*
Total Beverage Intake				
fl oz	111.2±106.9	108±42	3.7±9.0	0.413*
Kcal	454±452	314±249	140±36*	0.465*

[a]Validity determined by comparing second administration of the BEVQ-15 to averaged intake report from three 24-hr dietary recalls.
[b]Mean differences determined through paired t-test
*p≤0.05

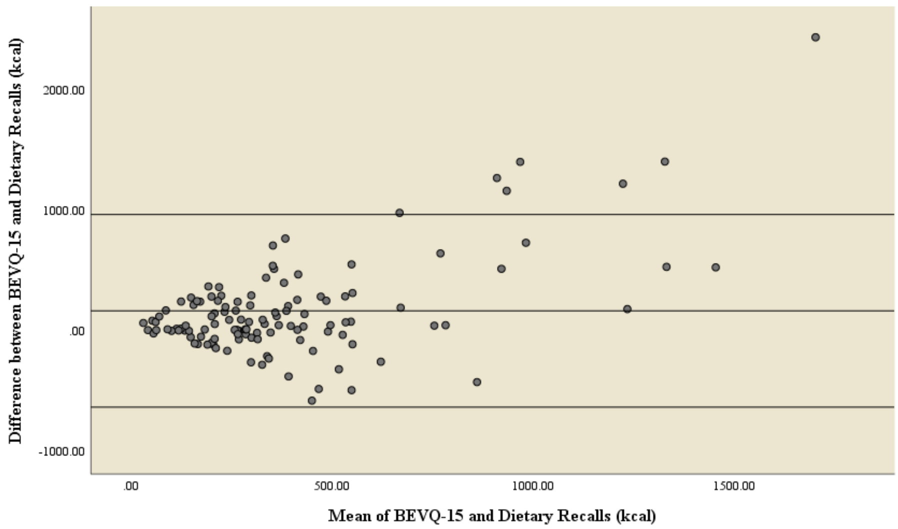
Table 3.3 Evaluation of Reliability of BEVQ-15

Beverage Category	Reliability[a]			
	BEVQ-15 (1)	**BEVQ-15 (2)**	**Difference[b]**	**Correlation (r)**
	←Mean±SD→		**Mean±SEM**	
Water (fl oz)	77.6±58.1	77.7±104.6	0±6	0.790*
100% Fruit Juice				
fl oz	2.7±5.3	2.3±5.8	0±1	0.354*
Kcal	48±94	40±104	7±10	0.354*
Sweetened Fruit Juice				
fl oz	1.7±5.1	1.6±5.5	0±0	0.479*
Kcal	25±72	23±78	2±7	0.479*
Whole Milk, Reduced Fat Milk, or Chocolate Milk				
fl oz	4.8±7.5	4.6±8.5	0±0	0.781*
Kcal	96±149	91±168	4±9	0.781*
Low Fat or Fat Free Milk, Buttermilk, or Soy Milk				
fl oz	1.7±5.2	1.9±4.7	0±-1	0.791*
Kcal	21±63	23±57	-2±4	0.791*
Nut Milk				
fl oz	1.1±5.3	1.0±2.8	0±-1	0.493*
Kcal	3±21	1±7	2±2	0.137
Regular Soft Drink				
fl oz	1.0±2.2	0.9±1.8	0±0	0.506*
Kcal	13±29	11±24	1±2	0.506*
Energy & Sports Drink				
fl oz	12.7±23.5	11±17	2±2	0.690*
Kcal	178±329	154±241	24±22	0.690*
Diet or Artificially Sweetened Soft Drink (fl oz)	0±2	0±2	0±0	0.294*
Sweet Tea				
fl oz	1±2	1±3	0±0	0.603*
Kcal	9±22	8±26	1±2	0.603*
Coffee/Tea – Black				
fl oz	2±5	2±5	0±0	0.557*
Kcal	1±4	1±6	0±1	0.013
Coffee/Tea – Sweetened/Milk/Cream				
fl oz	3±5	3±5	0±-1	0.816*
Kcal	15±31	16±33	-1±2	0.726*
Wine				
fl oz	0±1	0±1	0±0	0.842*
Kcal	6±22	6±22	0±1	0.842*
Hard Liquor				
fl oz	0±1	0±1	0±0	0.779*
Kcal	20±52	14±40	6±3	0.779*
Beer				
fl oz	1± 3	1± 3	0±0	0.754*
Kcal	10±26	10±28	0±2	0.754*
Total Sugar-Sweetened Beverages				
fl oz	19±25	16±22	2±-2	0.579*
Kcal	240±351	211±293	28±26	0.608*
Total Beverage Intake				
fl oz	115±72	112±107	3±-10	0.734*
Kcal	498±531	455±452	43±31	0.769*

[a]Reliability assessed by comparing the first and second administrations of the BEVQ-15
[b]Mean differences determined through paired t-test
*$p \leq 0.05$



(a)



(b)

Figure 3.3 Bland-Altman analysis of the BEVQ-15 and 24-hr Dietary Recalls. (a) Total beverage fl oz; (b) Total beverage kcal

URINALYSIS

There was a weak inverse relationship between USG and total beverage intake using the BEVQ-15 (fl oz) at each time point (r= -0.164 and r= -0.139). As would be expected, this indicated that as beverage intake increased, USG values decreased. Overall, approximately 70% of athletes were not adequately hydrated, based on UC and USG, prior to practice (Table 3.4). A majority of athletes classified themselves as mildly dehydrated at either urine collection, based on the UC chart. Sex differences were minimal and not significant; however, more males (73%) were dehydrated, prior to performing exercise than their female counterparts (66%), based on the UC chart. Of the 12 sports, 68% of cross country athletes presented the highest frequency of self-classified dehydration, based on their own UC. From the researcher's assessment of UC, 61% of cross country and 43% of swim team athletes were dehydrated. Lastly, based upon USG 87% of basketball and 80% of swim were inadequately hydrated prior to practice. On the other hand, self-classification of UC demonstrated that 59% of tennis and 57% of swim team members were properly hydrated for their sport. The researcher's assessment of UC determined that 62% soccer and 59% of tennis team members were properly hydrated. Lastly, based upon USG 27% of tennis was properly hydrated, resulting in this sport being the most hydrated of our sample population.

<u>Validity</u>

Associations were detected when comparing UC to USG, at the first time point (UC_{sub} $r=0.671$ and UC_{res} $r=0.805$, $p\leq0.05$). Similar results were found at the second time point (UC_{sub} $r=0.675$ and UC_{res} $r=0.884$, $p\leq0.05$). The subject's assessment of UC compared to USG demonstrated a significant level of agreement at each urine collection time point ($\kappa=0.192$ and $\kappa=0.181$, $p\leq0.05$, respectively). The researcher's assessment of UC compared to USG at each urine collection time point demonstrated similar results ($\kappa=0.267$ and $\kappa=0.260$, $p\leq0.05$, respectively). When comparing correlation coefficients (i.e. UC_{sub} to UC_{res}) at each time point, significant differences were noted ($z=-2.3$ and $z=-4.39$, $p\leq0.05$, respectively). Therefore, correlations were significantly higher for UC in researchers than in subjects, indicating greater validity.

Table 3.4 Evaluation of Validity for Urinalysis

Urine Assessment (1st Collection)	Total	Male	Female	Correlation (r)[a]
UC_{res}*	3.75 ± 0.15	3.93 ± 0.22	3.60 ± 0.22	
1-2, n(%)	31 (25.8)	13 (23.6)	18 (27.7)	0.805***
3-4, n(%)	48 (40.0)	22 (40.0)	26 (40.0)	
5-6, n(%)	41 (34.2)	20 (36.4)	21 (32.3)	
7-8, n(%)	0 (0.0)	0 (0.0)	0 (0.0)	
UC_{sub}	3.56 ± 0.14	3.67 ± 0.21	3.49 ± 0.18	
1-2, n(%)	33 (27.5)	14 (25.5)	19 (29.2)	
3-4, n(%)	61 (50.8)	27 (49.0)	34 (52.3)	0.671***
5-6, n(%)	26 (21.7)	14 (25.5)	12 (18.5)	
7-8, n(%)	0 (0.0)	0 (0.0)	0 (0.0)	
USG**	1.017 ± 0.001	1.018 ± 0.001	1.016 ± 0.001	
≤1.010, n(%)	25 (20.8)	9 (16.4)	16 (24.6)	
1.011-1.020, n(%)	53 (44.2)	24 (43.6)	29 (44.6)	
1.021-1.029, n(%)	39 (32.5)	21 (38.2)	18 (27.7)	
≥1.030, n(%)	3 (2.5)	1 (1.8)	2 (3.1)	
Urine Assessment (2nd Collection)	**Total**	**Male**	**Female**	**Correlation (r)[a]**
UC_{res}*	3.34 ± 0.15	3.44 ± 0.20	3.14 ± 0.20	
1-2, n(%)	41 (34.2)	15 (27.3)	26 (40.0)	
3-4, n(%)	49 (40.8)	27 (49.1)	22 (33.8)	0.884***
5-6, n(%)	28 (23.3)	13 (23.6)	15 (23.1)	
7-8, n(%)	2 (1.7)	0 (0.0)	2 (3.1)	
UC_{sub}	3.28 ± 0.14	3.45 ± 0.20	3.25 ± 0.23	
1-2, n(%)	42 (35.0)	16 (29.2)	26 (40.0)	
3-4, n(%)	55 (45.8)	29 (52.7)	26 (40.0)	0.675***
5-6, n(%)	21(17.5)	8 (14.5)	13 (20.0)	
7-8, n(%)	2 (1.7)	2 (3.6)	0 (0.0)	
USG**	1.016 ± 0.001	1.017 ± 0.001	1.016 ± 0.001	
≤1.010, n(%)	32 (26.6)	12 (21.9)	20 (30.8)	
1.011-1.020, n(%)	48 (40.0)	24 (43.6)	24 (36.9)	
1.021-1.029, n(%)	38 (31.7)	19 (34.5)	19 (29.2)	
≥1.030, n(%)	2 (1.7)	0 (0.0)	2 (3.1)	

[a]Validity assessed between both researcher and subject's assessment of UC to USG

*UC color ratings: 1-2 well-hydrated; 3-4 mildly dehydrated; 5-6 significantly dehydrated; 7-8 severely dehydrated

**USG ratings: <1.010 well hydrated; 1.010-1.020 minimal dehydration; 1.021-1.030 significant dehydration; >1.030 severe dehydration

***p≤0.05

Reliability

Mean urine color rating was UC_{sub} 3.4±1.5 and UC_{res} 3.5±1.7. UC ratings between subject and researcher were associated on both time points (r=0.821 and r=0.749, $p \leq 0.05$, respectively). Inter-rater reliability was demonstrated between the subject and researcher's assessment of UC at each time point (κ=0.407 and κ=0.224, $p \leq 0.05$, respectively). When comparing correlation coefficients, for test-retest reliability, between UC_{sub} and UC_{res}, there was no difference (z=1.45, p<0.15). Therefore, associations did not differ according to rater (i.e. subject vs researcher) at the two time points.

Table 3.4 Evaluation of Reliability for Urinalysis

Urine Assessment (1st Collection)	**Total**	**Male**	**Female**	**Correlation (r)[a]**
UC_{res}*	3.75 ± 0.15	3.93 ± 0.22	3.60 ± 0.22	0.821**
1-2, n(%)	31 (25.8)	13 (23.6)	18 (27.7)	
3-4, n(%)	48 (40.0)	22 (40.0)	26 (40.0)	
5-6, n(%)	41 (34.2)	20 (36.4)	21 (32.3)	
7-8, n(%)	0 (0.0)	0 (0.0)	0 (0.0)	
UC_{sub}	3.56 ± 0.14	3.67 ± 0.21	3.49 ± 0.18	
1-2, n(%)	33 (27.5)	14 (25.5)	19 (29.2)	
3-4, n(%)	61 (50.8)	27 (49.0)	34 (52.3)	
5-6, n(%)	26 (21.7)	14 (25.5)	12 (18.5)	
7-8, n(%)	0 (0.0)	0 (0.0)	0 (0.0)	
Urine Assessment (2nd Collection)	**Total**	**Male**	**Female**	**Correlation (r)[a]**
UC_{res}*	3.34 ± 0.15	3.44 ± 0.20	3.14 ± 0.20	0.749**
1-2, n(%)	41 (34.2)	15 (27.3)	26 (40.0)	
3-4, n(%)	49 (40.8)	27 (49.1)	22 (33.8)	
5-6, n(%)	28 (23.3)	13 (23.6)	15 (23.1)	
7-8, n(%)	2 (1.7)	0 (0.0)	2 (3.1)	
UC_{sub}	3.28 ± 0.14	3.45 ± 0.20	3.25 ± 0.23	
1-2, n(%)	42 (35.0)	16 (29.2)	26 (40.0)	
3-4, n(%)	55 (45.8)	29 (52.7)	26 (40.0)	
5-6, n(%)	21(17.5)	8 (14.5)	13 (20.0)	
7-8, n(%)	2 (1.7)	2 (3.6)	0 (0.0)	

[a] Reliability determined between researcher and subjects' assessment of UC at each time point
All values are expressed in Mean ± SEM
*UC color ratings: 1-2 well-hydrated; 3-4 mildly dehydrated; 5-6 significantly dehydrated; 7-8 severely dehydrated
**$p \leq 0.05$

DISCUSSION

This investigation evaluated the validity and reliability of two practical methods for assessing hydration status in collegiate athletes. Currently, there are no validated beverage consumption questionnaires to assess habitual beverage intake within the athletic population. With regard to total beverage intake, these findings indicate that the BEVQ-15 demonstrated the ability to provide valid and reliable information, regarding beverage consumption, compared to multiple dietary recalls within this population. Results of the correlational analyses were similar to what has been previously reported, in different populations.[15,17,19] In addition, Bland-Altman analysis further supported the agreement between these two assessment methods. These findings also suggest that UC can accurately assess hydration status within collegiate athletes. There was a fair to moderate agreement between USG and UC (subject and researcher's ratings) when assessing validity and reliability. Therefore, these practical methods for evaluating beverage intake and hydration status can be used in real-world collegiate athletic settings. To our knowledge, this is the first study to assess the validity of UC compared to USG within a variety of collegiate sports. However, previous research suggests that more education on proper hydration and understanding signs of dehydration could benefit all collegiate athletes and instill healthy hydration practices.[31]

With any dietary recall or food frequency questionnaire, there is under or over reporting error from the participants.[32] The validation of dietary assessment methods depends on the strength of the reference measure, in this case multiple 24-hr dietary recalls.[29] When conducting validation studies, it is recommended that methods be selected that do not have correlated errors. For this reason, 24-hour recalls are the preferred reference method for validating an FFQ. For instance, the use of the 24-hr recall captures recent intake of a food/beverage that may not have been reported on the BEVQ-15. However, the assessment of habitual beverage consumption compared to recent intake explains the differences in means and the strength of the correlations in this investigation. This study is consistent with previous research. The BEVQ-15 has the capability of capturing a more accurate representation of beverage consumption because it can measure frequency (i.e. less than 1 time per week) for each category.[17,19-22] A dietary recall may not capture the consumption of a specific beverage if a participant consumes it 1 time per week. Therefore, the Bland-Altman analysis demonstrates the slightly under an acceptable agreement between these two methods, with the standard being ≥95% agreement.[33,34] In addition, the correlations (r=0.297-0.686) between these two methods are consistent with previous research in children and adults.[15,17,19]

Previous literature suggests that the incorporation of sodium and potassium within foods and beverages, before exercise, will enhance the ability for the body to retain fluids.[35,36] Based on researcher-participant interactions, several modifications could potentially improve the validity of this questionnaire for the athletic population. However, more categories could increase the time to complete the questionnaire, which could be a disadvantage to the tool. Nonetheless, modifications could include separating energy and sports drinks (regular and diet), to more clearly distinguish between these categories, since some athletes commented or asked for clarification on this category. In addition, the specification of which type or brand of sports beverage is being consumed will more accurately determine the macronutrient and micronutrient beverage consumption for each participant.[12] Many athletes utilized the "other" category for writing in the additional beverages that they were regularly consuming. Therefore, another modification could include post-exercise recovery beverage options (i.e. protein powder, ready-to-go protein shakes), based on their BEVQ-15 reports. Research has shown that the incorporation of certain macronutrients (i.e. carbohydrates and protein) following exercise will enhance nutrient absorption and promote glycogen and protein synthesis in the body.[9,12,37] An additional modification could include separating chocolate milk from the other milk options and alternatives, by fat percentage (i.e. 1% and 2%). The consumption of chocolate milk has been shown to positively affect muscular strength and recovery in athletes.[38-40] These modifications could assist sports health practitioners and researchers to better assess the status of their athletes and provide more accurate and individualized recommendations.

An athlete should pay attention to the individual body cues such as UC, body weight changes, and thirst to avoid entering a dehydrated state prior to exercise.[41] Our results illustrate that NCAA Division 1 collegiate athletes, from a variety of sports, have the ability to self-assess their UC to the color chart. Difficulty arose for athletes when distinguishing between well hydrated and mildly dehydrated (i.e. between 3-5 on UC chart). Therefore, slight modifications could be made to allow athletes to better determine their hydration status. In addition, the positive relationship between UC and USG indicates that UC can accurately reflect the hydration status of a collegiate athlete. This is consistent with previous research conducted by Armstrong et al., in collegiate tennis athletes.[25] According to the NATA, an athlete's USG values <1.010 was classified as euhydrated, 1.010-1.020 was classified as dehydrated, and >1.020 was severely dehydrated.[28]

STRENGTH AND LIMITATIONS

Strengths of this investigation include a large sample size with a variety of different collegiate sports, both male and female, from two universities. Therefore, these results could be generalizable to other NCAA collegiate athletes, within this geographical region. Additionally, this investigation was the first to assess the validity and reliability of a beverage questionnaire within NCAA Division 1 collegiate athletes. To accurately assess validity of an assessment tool, it is important to have a large sample size.[42] The BEVQ-15 is a quantitative questionnaire that can be completed and scored quickly, which provides the ability to give feedback and recommendations on beverage intake for each participant was provided with a few days of completing the study. Additionally, the BEVQ-15 was compared to the "gold-standard" of self-reported dietary intake (i.e. multiple 24-hr recalls).[42] Lastly, UC is compared to an objective indicator of hydration status and can be utilized in a real-world setting to assess hydration status.

We acknowledge several limitations. Firstly, dietary recalls and questionnaires are self-reported, and thus potentially subject to under/over-reporting. Additionally, we did not control for other factors that could influence UC, such as medications, supplements, or menstrual cycle for female athletes.[43,44] Lastly, there was no timing protocol for collecting UC originally developed for this investigation due to the need to complete study sessions along with regular team practice schedules. The timing of collection (e.g. first morning void, second morning void, or 24-hr analysis) could have influenced the hydration status of the athletes.[45]

CONCLUSION

The BEVQ-15 demonstrated significant validity and reliability with the collegiate athletic population, when compared to multiple 24-hr dietary recalls. Future research could determine if modifications could improve the ability of the tool to capture habitual beverage consumption within this population. In addition, UC presented significant validity and reliability within collegiate athletes when compared to USG. This simple tool can help collegiate athletes self-assess their hydration status and enhance their overall performance. Together, these practical methods can be used in the collegiate athletic setting to assess athletes' hydration habits and status, and develop individual hydration plans. Future studies may be needed to reassess the validity of the modified BEVQ-15 and evaluate the sensitivity to change within this population.

REFERENCES

1. Spano M. KL, Thomas DT. Nutrition for Sport, Exercise and Health. 2018.
2. Popkin BM, D'Anci KE, Rosenberg IH. Water, hydration, and health. *Nutr Rev.* 2010;68(8):439-458.
3. Sawka MN, Burke, L. M., Eichner, E. R., et al. American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc.* 2007;39(2):377-390.
4. Jequier E, Constant F. Water as an essential nutrient: the physiological basis of hydration. *Eur J Clin Nutr.* 2010;64(2):115-123.
5. Hancock PA, Vasmatzidis I. Effects of heat stress on cognitive performance: the current state of knowledge. *Int J Hyperthermia.* 2003;19(3):355-372.
6. Binkley HM, Beckett J, Casa DJ, Kleiner DM, Plummer PE. National Athletic Trainers' Association Position Statement: Exertional heat illnesses. *J Athl Train.* 2002;37(3):329-343.
7. Castro-Sepulveda M, Cerda-Kohler H, Perez-Luco C, et al. Hydration status after exercise affect resting metabolic rate and heart rate variability. *Nutricion Hospitalaria.* 2015;31(3):1273-1277.
8. Dietary Reference Intake for Water, Potassium, Sodium, Chloride, and Sulfate. *THE NATIONAL ACADEMIES PRESS.* 2004.
9. Evans GH, Shirreffs SM, Maughan RJ. Postexercise rehydration in man: the effects of osmolality and carbohydrate content of ingested drinks. *Nutrition.* 2009;25(9):905-913.

10. Maughan RJ, Shirreffs SM. Rehydration and recovery after exercise. *Sci & Sport.* 2004;19(5):234-238.

11. Shirreffs SM, Maughan RJ. Volume repletion after exercise-induced volume depletion in humans: replacement of water and sodium losses. *Am J Physiol.* 1998;274(5 Pt 2):F868-875.

12. Thomas DT, Erdman KA, Burke LM. American College of Sports Medicine Joint Position Statement: Nutrition and athletic performance. *Med Sci Sports Exerc.* 2016;48(3):543-568.

13. Clarke MM, Stanhewicz AE, Wolf ST, Cheuvront SN, Kenefick RW, Kenney WL. A randomized trial to assess beverage hydration index in healthy older adults. *Am J Clin Nutr.* 2019;109(6):1640-1647.

14. Maughan RJ, Watson P, Cordery PA, et al. A randomized trial to assess the potential of different beverages to affect hydration status: development of a beverage hydration index. *Am J Clin Nutr.* 2016;103(3):717-723.

15. Hedrick VE, Savla J, Comber DL, et al. Development of a brief questionnaire to assess habitual beverage intake (BEVQ-15): sugar-sweetened beverages and total beverage energy intake. *J Acad Nutr Diet.* 2012;112(6):840-849.

16. Elwan D, Schweinitz P, Wojcicki JM. Beverage consumption in an Alaska Native village: a mixed-methods study of behaviour, attitudes and access. *Int J Circumpolar Health.* 2016;75(1):29905.

17. Hedrick VE, Comber DL, Ferguson KE, et al. A rapid beverage intake questionnaire can detect changes in beverage intake. *Eating behaviors.* 2013;14(1):90-94.

18. Hedrick VE, Myers EA, Zoellner JM, Duffey KJ, Davy BM. Validation of a rapid method to assess habitual beverage intake patterns. *Nutrients.* 2018;10(1).

19. Hill CE, MacDougall, C. R., Riebl, S. K., et al. Evaluation of the relative validity and test-retest reliability of a 15-Item beverage intake questionnaire in children and adolescents. *J Acad Nutr Diet.* 2017;117(11):1757-1766.e1755.

20. Hooper LP, Myers, E. A., Zoellner, J. M., et al. The impact of health literacy status on the comparative validity and sensitivity of an interactive multimedia beverage intake questionnaire. *Nutrients.* 2016;9(1).

21. Lora KR, Davy, B., Hedrick, V., et al. Assessing initial validity and reliability of a beverage intake questionnaire in hispanic preschool-aged children. *J Acad Nutr Diet.* 2016;116(12):1951-1960.

22. Riebl SK, Paone, A. C., Hedrick, V. E., et al. The comparative validity of interactive multimedia questionnaires to paper-administered questionnaires for beverage intake and physical activity: pilot study. *JMIR Res Protoc.* 2013;2(2):e40.

23. Davy BM, You W, Almeida F, et al. Impact of individual and worksite environmental factors on water and sugar-sweetened beverage consumption among overweight employees. *Prev Chronic Dis.* 2014;11:E71.

24. Armstrong LE. Assessing hydration status: the elusive gold standard. *J Am Coll Nutr.* 2007;26(5 Suppl):575S-584S.

25. Armstrong LE, Maresh CM, Castellani JW, et al. Urinary indices of hydration status. *Int J Sport Nutr.* 1994;4(3):265-279.

26. AMPM-USDA Automated Multiple Pass Method. https://www-ars-usda-gov.ezproxy.lib.vt.edu/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-

research-center/food-surveys-research-group/docs/ampm-usda-automated-multiple-pass-method/.

27. Moshfegh AJ, Rhodes DG, Baer DJ, et al. The US Department of Agriculture automated multiple-pass method reduces bias in the collection of energy intakes. *Am J Clin Nutr.* 2008;88(2):324-332.

28. Casa DJ, Armstrong LE, Hillman SK, et al. National athletic trainers' association position statement: fluid replacement for athletes. *J Athl Train.* 2000;35(2):212-224.

29. Kirkpatrick SI, Baranowski, T., Subar, A. F., et al. Best practices for conducting and interpreting studies to validate self-report dietary assessment methods. *J Acad Nutr Diet.* 2019;119(11):1801-1816.

30. Mukaka MM. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. *Malawi Med J.* 2012;24(3):69-71.

31. Volpe SL, Poule KA, Bland EG. Estimation of prepractice hydration status of National Collegiate Athletic Association Division I athletes. *J Athl Train.* 2009;44(6):624-629.

32. Ortega RM, Perez-Rodrigo C, Lopez-Sobaler AM. Dietary assessment methods: dietary records. *Nutr Hosp.* 2015;31 Suppl 3:38-45.

33. Dogan NO. Bland-Altman analysis: A paradigm to understand correlation and agreement. *Turk J Emerg Med.* 2018;18(4):139-141.

34. Giavarina D. Understanding Bland Altman analysis. *Biochem Med.* 2015;25(2):141-151.

35. Merson SJ, Maughan RJ, Shirreffs SM. Rehydration with drinks differing in sodium concentration and recovery from moderate exercise-induced hypohydration in man. *Eur J Appl Physiol.* 2008;103(5):585-594.

36. Armstrong LE, Costill DL, Fink WJ, et al. Effects of dietary sodium on body and muscle potassium content during heat acclimation. *Eur J Appl Physiol Occup Physiol.* 1985;54(4):391-397.

37. Areta JL, Burke LM, Ross ML, et al. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *J Physiol.* 2013;591(9):2319-2331.

38. Amiri M, Ghiasvand R, Kaviani M, Forbes SC, Salehi-Abargouei A. Chocolate milk for recovery from exercise: a systematic review and meta-analysis of controlled clinical trials. *Eur J Clin Nutr.* 2019;73(6):835-849.

39. Born KA, Dooley EE, Cheshire PA, et al. Chocolate milk versus carbohydrate supplements in adolescent athletes: a field based study. *J Int Soc Sports Nutr.* 2019;16(1):6.

40. Marakis G, Kontopoulou, L., Garofalakis, G., et al. Development and relative validity of a self-administered semi-quantitative drinks frequency questionnaire, validated in a population of university students. *Nutr Diet.* 2019;76(5):532-538.

41. McDermott BP, Anderson SA, Armstrong LE, et al. National Athletic Trainers' Association Position Statement: Fluid replacement for the physically active. *J Athl Train.* 2017;52(9):877-895.

42. Van Horn L. BJ. *Research: Successful Approaches in Nutrition and Dietetics.* 4th ed: Acad Nutr Diet; 2019.

43. Armstrong LE, Johnson EC, Munoz CX, et al. Hydration biomarkers and dietary fluid consumption of women. *J Acad Nutr Diet.* 2012;112(7):1056-1061.

44. Armstrong LE, Soto JA, Hacker FT, Jr., Casa DJ, Kavouras SA, Maresh CM. Urinary indices during dehydration, exercise, and rehydration. *Int J Sport Nutr.* 1998;8(4):345-355.

45. Armstrong LE, Pumerantz AC, Fiala KA, et al. Human hydration indices: acute and longitudinal reference values. *Int J Sport Nutr Exerc Metab.* 2010;20(2):145-153.

CHAPTER 4: THE VALIDITY OF URINE COLOR AS A HYDRATION BIOMARKER WITHIN THE GENERAL ADULT POPULATION: A SYSTEMATIC REVIEW

ABSTRACT

Frequent monitoring of hydration status can help to avoid the adverse effects of dehydration, such as impairments in physical performance and cognitive function. Methods used to measure hydration include plasma/serum osmolality, urinary specific gravity (USG), urine osmolality (Uosm), change in body weight, urine volume, and urine color. With the exception of urine color assessment, most methods are impractical for the general population to use on a day-to-day basis. However, the validity of this method has not been systematically evaluated. The objective of this systematic review is to determine if the assessment of urine color is a valid method for determining hydration status in the general adult population, including older adults and athletes. Using the PRISMA guidelines, electronic databases were searched to identify original research articles of all study design types for inclusion. Of the 424 articles screened, 11 met inclusion criteria. Most studies compared urine color to either USG or Uosm, and reported significant associations (r) ranging from 0.35 to 0.93. Lower correlation coefficients were noted in studies of older adults from 60-101 years old. Studies generally reported a high sensitivity of urine color as a diagnostic tool for detecting dehydration, and the ability to distinguish across categories of hydration status. In 10 of 11 studies, urine color was evaluated by a researcher; therefore, the validity of this method for determining daily hydration status in real-world conditions is uncertain. Future research is needed to address this issue and extend these findings to other populations, such as children and youth athletes.

Keywords: hydration status, urine color, adult, validity

INTRODUCTION

Maintaining adequate fluid consumption and hydration status are crucial for normal physiological functions[1,2], as dehydration can cause adverse health effects.[2,3] Mild dehydration (i.e., 1-2% body water loss) can impair memory and concentration, and lead to headaches and anxiety.[4] Hypohydration can have more severe effects.[5] For example, losing ≥ 2% body weight can cause cognitive impairment, fatigue, increased cardiovascular strain, and increased heat-stroke risk.[1,4,6] For individuals engaging in high-intensity exercise, such as athletes, hypohydration can result in muscle cramps, acute renal failure, and heat illness.[4,7] Therefore, hydration status assessment methods, which can feasibly be used on a daily basis in real-world settings, are needed to help avoid these adverse effects.

Techniques that can be used to determine hydration status include plasma/serum osmolality, urinary specific gravity (USG), urine osmolality (Uosm), changes in body weight, urine volume, and urine color.[4] Although serum osmolality is considered the gold standard for detecting acute changes in hydration status[7], it is not practical for daily measurement. Urinary indices, such as USG, urine color, and Uosm, are less invasive, more feasible, and more cost-effective for assessing day-to-day hydration.[8,9] Uosm and USG, methods that determine solute particle concentrations and are validated methods for measuring hydration status. However, these techniques require trained assessors and are thus less feasible for real-world settings.[9,10]

Urine color and urine volume, which are the least expensive methods, can be easily self-evaluated[8,11], and have been validated in athletes and children.[8,9,12,13] While urine volume is an objective indicator of hydration status, it can be challenging to utilize in free-living situations due to urine collection requirements. Armstrong et al., developed an eight scale urine color chart, that has been widely utilized.[9,12-14] Each color corresponds with a number; 1-3 well hydrated, 4-6 moderately dehydrated, 7-8 severely dehydrated.[9] To our knowledge, no systematic reviews have evaluated the validity of urine color as a hydration assessment method in the general adult population, including athletes and older adults. Therefore, the objective of this systematic review is to determine if urine color assessment is a valid method for determining hydration status, and to identify future research needs.

METHODS

STUDY EXCLUSION AND INCLUSION CRITERIA

Eligibility criteria were developed using the population, intervention, comparison, outcome, and study design (PICOS) model, which is presented in Table 4.1.

Table 1. PICOS Objectives

Population	General Adult Population (18+ years old), including athletes
Intervention	Evaluation of hydration status
Comparison	Other validated indicators of hydration status; evaluate the quality of evidence
Outcomes	Validity and reliability of urine color as a hydration status indicator
Study Design	RCT, Quasi-experimental, observational, cross-sectional

This review included clinical trials, randomized controlled trials (RCT), experimental studies, quasi-experimental designs, observational, cohort, and cross-sectional studies that were published before May 2019. Meta-analyses, systematic reviews, narrative reviews, and nonsystematic reviews were not included. Animal studies and studies that were conducted in children or adolescents were excluded. “General” adult population was defined as being free from chronic disease conditions that would impact hydration status. For example, individuals with impaired renal function may have alterations in urine color due to the disease process[4,7], and individuals with some chronic conditions, such as congestive heart failure or renal failure, may be prescribed fluid restrictions. This review was registered with PROSPERO (approval pending).

DATABASE SEARCH STRATEGIES

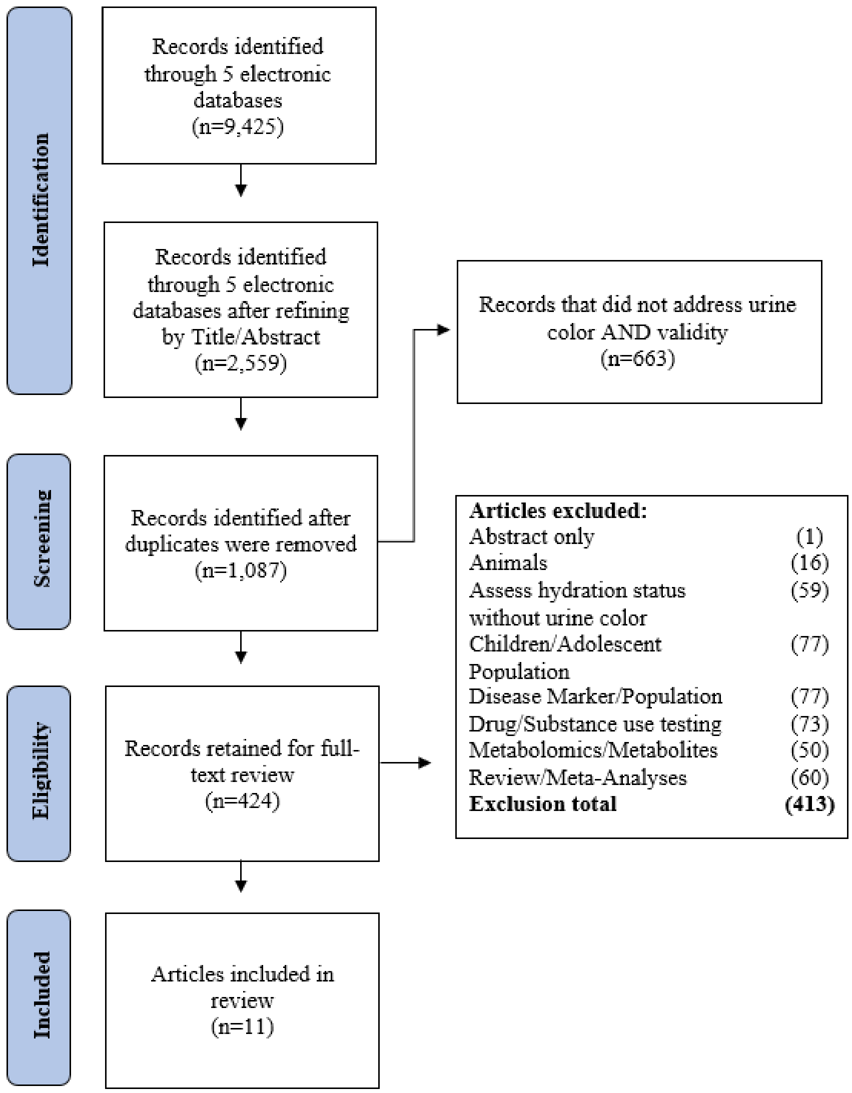
The Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines were used to develop search strategies.[15] Databases used for this systematic review include PubMed from NLM (1946-present); Medline from ProQuest (1946-present); Cumulative

Index of Nursing and Allied Health Literature (CINAHL) from EbscoHost (1981-present); Scopus (1800s-present); and Web of Science Core Collection from Clarivate Analytics (1900-present). In total, there were 20 search terms and Boolean search operators used for each database, which are listed in Table 4.2.

Table 2. Search Term Combinations

Urinary Terms	Assessment	Population
Urine color AND:	Validity AND:	Adult
Urine AND:	Validation Study AND:	Older Adult
Urinalysis AND:	Reliability AND:	Young Adult
Urine Osmolality AND:	Reproducibility AND:	College Student
Plasma Osmolality AND:		Athlete
Fluid Balance AND:		
Hydration AND:		
Hydration Status AND:		
Euhydration AND:		
Dehydration AND:		
Hypohydration AND:		

Following the initial search stage, articles were imported into Endnote and title and abstracts were reviewed for inclusion/exclusion criteria. The search strategy and abstract review process was duplicated by a second reviewer; the two reviewers met to resolve differences in outcomes of the search and abstract review process. Articles which appeared to meet the established eligibility requirements underwent full-text review (n=424). Figure 4.1 presents an overview of the search process and criteria for article exclusion.



For included articles, article quality ratings which included risk of bias were performed independently by two reviewers (S.K. and B.D.) using the Academy of Nutrition and Dietetics Evidence Analysis Library Quality Rating Tool.[16] Quality Rating outcome was defined as Positive (most answers rated "yes"); Neutral; Negative ($\geq$ 6 answers rated "no"). Each of the validity questions contained multiple specific questions to better determine the overall answer ("Yes", "No", "Uncertain", "N/A"). An "uncertain" answer means that the research did not distinctly state or explain a concept enough to receive a "yes" or "no." A Neutral rating suggests that the research was neither strong nor weak and is based on 4 of the validity questions within the Quality Rating Tool. These 4 questions include the “selection of the study subjects/patients free from bias,” “study groups comparable,” “intervention/therapeutic regimens/exposure factors or procedure and any comparison described in detail,” and “outcomes clearly defined and the measurements valid and reliable.” If these questions did not receive a “yes” then the overall rating was Neutral.[16]

RESULTS

Eleven articles were identified for inclusion in this review. Findings are summarized within the text according to method of comparison with urine color: USG, Uosm, serum osmolality (Sosm), and detailed in Table 4.3 according to study population. Two articles were included that were not designed as validation studies. However, urine color was either compared across categories of hydration status or across experimental conditions of dehydration vs hydration. As such, these studies could provide information on validity.[17,18]

Table 3. Summary of urine color studies in healthy adults (Before May 2019)						
Reference	Population (Sample Size)	Urine Color Procedure	Who Assessed Urine Color	Comparison Biomarker	Validity (p value)	Sensitivity/Specificity
Athletes						
Armstrong et al. (1994)	NCAA D1 Tennis Teams (8 female, 12 male)	Armstrong Urine Color Chart	Researcher	U_{sg}	r=0.78 (<0.0001)	
Armstrong et al. (1998)	Male Cyclists (n=9)	Armstrong Urine Color Chart	Researcher	U_{osm}	r=0.72 (<0.001)	
				U_{sg}	r=0.68 (<0.001)	
Harvey et al. (2007)	Premier Division Soccer Athletes (n=13)	Adapted Armstrong Urine Color Chart; used as a predictor of sweat loss during a soccer match (compared to other predictors)	Researcher	Sweat Loss	Best model included body mass loss + urine color change, $r^2 = 0.57$	
Fernandez-Elias et al. (2014)	Olympic Combat Athletes (244 male; 101 female)	Armstrong Urine Color Chart; ratings compared across three categories of hydration status (G1-G3)	Researcher	G1[a] U_{osm}	r=0.70 (<0.001)	
				G2[b] U_{osm}	r=0.50 (<0.002)	
				G3[c] U_{osm}	r=0.40 (<0.004)	
Older Adults (≥ 65 years)						
Mentes et al. (2006)	Nursing Home Residents (n=98; subgroup n=78)	Armstrong Urine Color Chart	Researcher	U_{sg}	r=0.48 (<0.01)	
Hooper et al. (2016)	Older Adults in DRIE (n=162)	Human Hydration LLC; www. hydrationcheck.com Diagnostic accuracy of assessing dehydration	Researcher	S_{osm}		ROC AUC=0.51, ≤ 70%
Pregnant Women						

McKenzie et al. (2017)	Pregnant (n=18) & Non-pregnant women (n=18)	Armstrong Urine Color Chart; Diagnostic accuracy of assessing $U_{osm} \geq 500$mOsm/kg	Researcher	U_{osm}	r=0.61-0.84 (<0.0001)	92% Preg; 96% Non-Preg/41% Preg; 80% Non-Preg (24-hr samples)
				U_{sg}	r=0.62-0.90 (<0.0001)	
College Students						
Armstrong et al. (1994)	College Males (n=23)	Armstrong Urine Color Chart	Researcher	U_{osm}	r=0.62 (<0.01)	
				U_{sg}	r=0.54 (<0.01)	
	College Males (n=11)	Armstrong Urine Color Chart	Researcher	U_{osm}†	r=0.92 (<0.001)	
				U_{sg}†	r=0.93 (<0.000001)	
Zhang et al. (2017)	College students in China (n=68, all males)	CIE Method[d] (L*a*b*); Diagnostic accuracy for assessing dehydration	Researcher (using a chromatrogram spetrophotometer)	L* U_{osm}	r=-0.56 (<0.0001)	
				b* U_{osm}	r=0.86 (<0.0001)	For b*: 97.4%/65.5%
				a* U_{osm}	r=-0.35 (<0.0001)	
Early et al. (2018)	College Students (n=16)	Armstrong Urine Color Chart; ratings compared on days 1, 2, 3 of each study period	Participant	Experimentally induced short-term (3 d.) hydration vs dehydration	Day 1: (p=0.04) Day 2: (p=0.002) Day 3: (p<0.001)	
General Population						
Malisova et al. (2016)	Healthy Adults from 3 different countries (n=573)	Armstrong Urine Color Chart; ratings compared across three categories of hydration status	Researcher	U_{sg}	p<0.001	
Perrier et al. (2017)	Healthy French Adults (n=82)	Armstrong Urine Color Chart; Diagnostic accuracy for assessing $U_{osm} > 500$mOsm/kg	Participant	U_{osm}		87.8%/64.3%

† pre-exercise comparison
(◊) significance not reported for comparison
[a] G1: Group 1 (euhydrated (250 - 700 mOsm·kg H_2O^{-1}))
[b] G2: Group 2 (dehydrated (700 - 1.080 mOsm·kg H_2O^{-1}))
[c] G3: Group 3 (severely dehydrated (1.080 - 1.500 mOsm·kg H_2O^{-1}))
[d] CIE (Commission Internationale de l'Eclarige) Method

URINARY SPECIFIC GRAVITY (USG)

Studies which compared urine color to USG utilized a variety of testing instruments, which included a Chemstrip Mini Urine Analyzer, Bayer's Multistix, an optical refractometer, and a hand-held refractometer. USG hydration categories are as follows: $\leq$1.013 is over-hydrated or hyper-hydrated; l.014-1.029 is euhydrated; $\geq$1.030 is dehydrated for the general population.[9] However, these categories are different for the athletic population, according to the National Athletic Trainers Association with 1.010-1.020 being dehydrated.[8,11] Armstrong et al., reported moderate to strong correlations between USG and urine color in the athletic population (r=0.68 – 0.78), and in college males (r=0.54 - 0.93) (9). Correlations similar in magnitude were reported by McKenzie et al. in pregnant and non-pregnant women (r=0.62-0.90).[19] In older adults, correlation coefficients between USG and urine color were substantially lower (Table 4.3). Therefore, urine color may better reflect hydration status in younger than in older populations.

URINE OSMOLALITY (UOSM)

Among urinary indicators of hydration status, Uosm is considered the most valid technique in athletes and healthy adults.[20-22] Of the studies reviewed, eight utilized Uosm as the comparator (Table 4.3). Moderate to high correlation coefficients (i.e., 0.6-0.9) between urine color and Uosm were reported in college-aged males[9] and young adult athletes[10] as well as in pregnant and non-pregnant women[19]. Urine color demonstrated a high sensitivity for detecting Uosm>500mOsm/kg in pregnant and non-pregnant women, and in healthy French adults (using urine color >4), but lower specificity in pregnant women (41%)[19] and healthy French adults (64.3%).[22] Although a spectrophotometer was used to assess three aspects of urine color in the study by Zhang et al.[23], a high sensitivity for detecting dehydration was noted.[24]

Two studies evaluated urine color compared to three categories of hydration status determined using Uosm; one in Olympic combat athletes[25] and one in healthy adults.[17]

Fernandez-Elias et al., determined three cutoff points based on Uosm as follows: euhydration (250-700 mOsm·kg H_2O^{-1}), mild dehydration (701-1.080 mOsm·kg H_2O^{-1}), and severe dehydration (1.081-1.500 mOsm·kg H_2O^{-1}).[25] Stronger correlations were noted between urine color and Uosm-determined euhydration and mild dehydration, than severe dehydration (Table 4.3). Malisova et al., assessed the hydration status in the general population of three different countries over a period of seven days during the summer and winter months and included the measurement of USG, Uosm, urine color and other urinary markers.[17] Uosm was used to characterize individuals as euhydrated or dehydrated. Urine color was significantly different ($P<0.001$) and consistent with classified hydration state across categories in both females (3.0 ± 1.2, 3.9 ± 1.2, 5.5 ± 1.2, respectively) and males (3.6 ± 1.4, 4.3 ± 1.1, 5.9 ± 1.0, respectively). Therefore, urine color may be a useful diagnostic tool for assessing dehydration or suboptimal hydration in a variety of populations including athletes and the general adult population, and for characterizing hydration status across a range of hydration levels.

SERUM OSMOLALITY (SOSM)

Although more invasive, Sosm can be used to detect minute changes in hydration status.[20] Reference intervals used to classify hydration status based on Sosm are as follows: 275 to < 295 mOsm/kg indicates euhydration, 295-300 mOsm/kg is mild dehydration, and >300 mOsm/kg is dehydration.[26] Only one study reviewed utilized this method of comparison; the Dehydration in our Elders (DRIE) study was conducted to assess the signs of water loss in older adults.[27] Hooper et al., found that less than half of the study participants were dehydrated according to Sosm. However, the sensitivity and specificity of urine color for assessing hydration as compared to Sosm for each group was suboptimal at ≤ 70%[27] suggesting that urine color should not be used to detect dehydration in aged adults.[27]

OTHER METHODS OF COMPARISON

Two studies reviewed utilized other methods of evaluating urine color as a hydration status indicator. Early et al., conducted a randomized crossover study in college students to determine the relationship between hydration status, cardiovascular (CV) function and thermoregulatory response (Table 4.3).[18] During the hydration phase, participants were asked to drink ample amounts of fluids and evaluate their urine color against the Armstrong urine color chart for three days.[10] The instruction was to maintain urine color at less than three on the eight scale urine color chart. During the dehydration phase, participants were asked to restrict their fluid intake so that their perceived urine color was greater than four on the urine color chart, for three days. Urine color ratings were compared on days 1, 2, and 3 of each phase, and were found to be significantly different (all $p<0.05$).

Harvey et al. evaluated predictors of sweat loss before and after soccer matches in Premier Division soccer players.[13] Predictors examined included urine color, USG, body mass, and hematocrit. The best prediction model included body mass change and urine color change ($r^2 = 0.57$). However, the investigators concluded that urine color alone does not adequately assess hydration status. Therefore, urine color changes may be a suitable method for determining hydration status leading up to important competition and training, but not pre and post event.

ARTICLE QUALITY ASSESSMENT

Results of the article quality assessment are provided in Table 4.4. Based on the criteria previously mentioned, there was 100% agreement between reviewers. Of the articles evaluated, 9 were judged to be of positive overall quality and 2 were judged to be of neutral overall quality. On the Quality Criteria Checklist, question 5 of the validity questions ("was blinding used to prevent introduction of bias") was the most problematic across all studies. Overall study quality was generally positive.[16]

Table 4.4 Article Quality Assessment

Author, year	1. Research question clearly stated	2. Participant selection free from bias	3. Study groups comparable	4. Method of handling withdrawals described	5. Blinding used to prevent bias	6. Interventions or exposures described in detail	7. Outcomes defined, valid and reliable	8. Statistical analysis appropriate	9. Conclusions supported by results	10. Bias due to funding unlikely	Overall rating
Armstrong, 1998	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Yes	Yes	Neutral
Harvey, 2007	Yes	Yes	Yes	Unclear	No	Yes	Yes	Yes	Yes	Unclear	Positive
Fernandez, 2014	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Positive
Mentes, 2006	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Positive
Hooper, 2016	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Positive
McKenzie, 2017	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Positive
Armstrong, 1994	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Yes	Yes	Yes	Positive
Zhang, 2017	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Positive
Early, 2018	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Positive
Malisova, 2016	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Yes	Yes	Yes	Positive
Perrier, 2017	Yes	Unclear	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Neutral

Overall rating: Negative (-), Neutral (Θ), Positive (+). Of 10 criteria: 6 or more questions rated as "no" = (-); Most answers including 2,3,6,7 are "yes" = (+); If 2,3,6,7 are not "yes" = Θ.

DISCUSSION

This review evaluated literature that generally supported the validity of urine color as a measure of hydration status in the healthy adult population. However, the available research which has addressed the validation of urine color as a hydration biomarker is limited. The majority of the studies reviewed were conducted in the general adult population (8 studies; 2 in older adults, 1 in pregnant women); 4 studies were conducted in the athletic population, with 1 study overlapping between the two populations (see Table 4.3). Of the 11 articles identified, 9 evaluated the validity of urine color compared to previously established valid methods of hydration status. The other 2 articles identified predictors of urine color and evaluated sensitivity to change in urine color based upon manipulation of fluid intake over 3 days.[17,18]

Nine of the studies included in this review used the validated urine color chart developed by Armstrong and colleagues.[9] The studies that used already validated urinary indices (i.e., USG and Uosm) in both athletic and general adult populations found significant correlations when compared with urine color.[7, 9, 10, 19, 23, 25] Correlation coefficients were interpreted as follows: weak (r = 0.30-0.49), moderate (r = 0.50-0.69), strong (r = 0.70-1.00).[28] Three of the studies that compared urine color to another biomarker relied on different statistical methods such as the AIC Criterion and sensitivity/specificity.[13,22,27] These studies indicate that urine color is a valid assessment of hydration status; however, there are limitations when evaluating urine color as a sole hydration indicator.[22,23] There are a number of different factors that can influence urine color. These include acute changes in body mass[29,30], certain foods, medications and dietary supplements[31] and menstrual cycle phase.[10,32] Taking these limitations into consideration when conducting research is important when determining hydration status. Overall, the evidence supporting the validity of urine color as a hydration biomarker in the healthy adult population was relatively consistent and positive in quality.

There are several notable strengths of this review. First, an evidence-based framework was used to develop the research question (i.e., PICOS) and methodology. Second, the PRISMA guidelines, the recommended approach for designing and reporting the systematic review process, were utilized. Third, article quality ratings were performed independently by two evaluators. Lastly, the validated Armstrong urine color chart was utilized in nine of the eleven articles included in this review. The tool is a widely-available tool and could be implemented in both clinical and field settings.

There are several limitations in this body of literature that were identified. First, there were a small number of articles available for inclusion. Second, limitations in study methods related to validation methods across studies were noted (i.e., not every study assessed the validity of the urine color chart using a validated hydration biomarker). Thus, more research is needed to evaluate associations of these measures of hydration status in the healthy adult population. Most studies (10 of 11) relied on research staff for urine color assessment; therefore, the validity of this method for determining daily hydration status in real-world conditions is uncertain. Additionally, there could have been researcher bias of the color interpretation results. Limitations in article quality were also noted, although most studies were determined to be of positive quality.

CONCLUSION

This evaluation of the utilization of urine color as a hydration biomarker is an important area for investigation. However, there are a limited number of studies in this area that have been conducted in the healthy adult population as well as in athletes whose performance may depend on adequate hydration. Nonetheless, based on articles reviewed, urine color appears to be a reasonably valid and practical method of determining hydration status in the healthy adult population and athletes. Furthermore, future research should utilize Sosm, the gold standard, to evaluate the validity of urine color as a hydration assessment method.[21,33] In addition, future research is also needed to determine if individuals are able to adequately self-evaluate hydration status using this method and to determine if this method is valid in other populations such as children.

REFERENCES

1. Jequier E, Constant F. Water as an essential nutrient: the physiological basis of hydration. Eur J Clin Nutr. 2010;64(2):115-23.
2. Popkin BM, D'Anci KE, Rosenberg IH. Water, hydration, and health. Nutr Rev. 2010;68(8):439-58.
3. Hancock PA, Vasmatzidis I. Effects of heat stress on cognitive performance: the current state of knowledge. Int J Hyperthermia. 2003;19(3):355-72.
4. Sawka MN, Burke LM, Eichner ER, Maughan RJ, et al. American College of Sports Medicine position stand. Exercise and fluid replacement. Med Sci Sports Exerc. 2007;39(2):377-90.
5. Spano M. KL, Thomas DT. Nutrition for Sport, Exercise and Health. 2018.
6. Arnaoutis G, Kavouras SA, Angelopoulou A, et al. Fluid balance during training in elite young athletes of different sports. J Strength Cond Res. 2015;29(12):3447-52.
7. Mentes JC, Wakefield B, Culp K. Use of a urine color chart to monitor hydration status in nursing home residents. Biol Res Nurs. 2006;7(3):197-203.
8. Casa DJ, Armstrong LE, Hillman SK, et al. National athletic trainers' association position statement: fluid replacement for athletes. J Athl Train. 2000;35(2):212-24.
9. Armstrong LE, Maresh CM, Castellani JW, et al. Urinary indices of hydration status. Int J Sport Nutr. 1994;4(3):265-79.
10. Armstrong LE, Soto JA, Hacker FT, et al. Urinary indices during dehydration, exercise, and rehydration. Int J Sport Nutr. 1998;8(4):345-55.
11. Thomas DT, Erdman KA, Burke LM. American College of Sports Medicine Joint Position Statement. Nutrition and Athletic Performance. Med Sci Sports Exerc. 2016;48(3):543-68.

12. Kavouras S, Johnson E, Bougatsas D, et al. Validation of a urine color scale for assessment of urine osmolality in healthy children. Eur J Nutr. 2016;55(3):907-15.

13. Harvey G, Meir R, Brooks L, Holloway K. The use of body mass changes as a practical measure of dehydration in team sports. J Sci Med Sport. 2008;11(6):600-3.

14. Culp K, Mentes J, Wakefield B. Hydration and acute confusion in long-term care residents. West J Nurs Res. 2003;25(3):251-66.

15. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med. 2009;6(7):e1000100.

16. Evidence Analysis Manual: Quality Criteria Checklist-Primary Research. Acad Nutr Diet: EAL; 2018.

17. Malisova O, Athanasatou A, Pepa A, et al. Water intake and hydration indices in healthy european adults: the european hydration research study (EHRS). Nutrients. 2016;8(4):204.

18. Early KS, Earnest CP, Theall B, et al. Free-living, continuous hypo-hydration, and cardiovascular response to exercise in a heated environment. Physiol Rep. 2018;6(8).

19. McKenzie A, Armstrong L, Muñoz C, Ellis L, et al. Urine color as an indicator of urine concentration in pregnant and lactating women. Eur J Nutr. 2017;56(1):355-62.

20. Popowski LA, Oppliger RA, Patrick Lambert G, et al. Blood and urinary measures of hydration status during progressive acute dehydration. Med Sci Sports Exerc. 2001;33(5):747-53.

21. Shirreffs SM, Maughan RJ. Urine osmolality and conductivity as indices of hydration status in athletes in the heat. Med Sci Sports Exerc. 1998;30(11):1598-602.

22. Perrier ET, Bottin JH, Vecchio M, Lemetais G. Criterion values for urine-specific gravity and urine color representing adequate water intake in healthy adults. Eur J Clin Nutr. 2017;71(4):561-3.

23. Zhang N, Du SM, Zheng MQ, et al. Urine color for assessment of dehydration among college men students in Hebei, China - a cross-sectional study. Asia Pac J Clin Nutr. 2017;26(5):788-93.

24. Takatsui F, Andrade MF, Neisser MP, et al. CIE L*a*b*: comparison of digital images obtained photographically by manual and automatic modes. Braz Oral Res. 2012;26(6):578-83.

25. Fernandez-Elias VE, Martinez-Abellan A, Lopez-Gullon JM, et al. Validity of hydration non-invasive indices during the weightcutting and official weigh-in for Olympic combat sports. PloS one. 2014;9(4):e95336.

26. Cheuvront SN, Ely BR, Kenefick RW, Sawka MN. Biological variation and diagnostic accuracy of dehydration assessment markers. Am J Clin Nutr. 2010;92(3):565-73.

27. Hooper L, Bunn DK, Abdelhamid A, et al. Water-loss (intracellular) dehydration assessed using urinary tests: how well do they work? Diagnostic accuracy in older people. Am J Clin Nutr. 2016;104(1):121-31.

28. Mukaka MM. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. Malawi Med J. 2012;24(3):69-71.

29. O'Connell BN, Weinheimer EM, Martin BR, et al. Water turnover assessment in overweight adolescents. Obesity. 2011;19(2):292-7.

30. Chang T, Ravi N, Plegue MA, et al. Inadequate Hydration, BMI, and Obesity Among US Adults: NHANES 2009-2012. Ann Fam Med. 2016;14(4):320-4.

31. Simerville JA, Maxted WC, Pahira JJ. Urinalysis: a comprehensive review. Am Fam Physician. 2005;71(6):1153-62.

32. Armstrong LE, Johnson EC, Munoz CX, et al. Hydration biomarkers and dietary fluid consumption of women. J Acad Nutr Diet. 2012;112(7):1056-61.

33. Hustrini NM, Siregar P, Nainggolan G, et al. Diagnostic performance of afternoon urine osmolality to assess optimal hydration status in an adult healthy population. Acta medica Indonesiana. 2017;49(2):112-7.

CHAPTER 5: CONCLUSION & FUTURE DIRECTIONS

The main findings of this dissertation highlighted the importance of monitoring beverage consumption and hydration status for both the general adult population and the collegiate athletic population. Previous studies have evaluated and validated beverage intake assessment tools within several populations. However, currently no beverage questionnaire has been validated within an athletic population. This research addressed this gap in the literature. We determined that the BEVQ-15 was valid and reliable for assessing habitual beverage intake among Division 1 athletes, in various NCAA collegiate sports. This tool demonstrated the ability to assess hydration status based on the frequency and amount of beverages consumed. Specific beverages have greater rehydration properties, including whey-permeate based beverages, due to their nutrient composition.[1] This research evaluated whey-permeate based hydration beverage formulations for acceptability and sensory attributes. Results of the study highlighted the valuable combination of nutrients provided for hydration by these beverages. However, it was determined that in order for these beverages to be market-ready, several formulation modifications are still needed to improve the acceptability for potential commercial success. Lastly, urine color (UC) yielded valid results when compared to urinary specific gravity (USG) in NCAA Division 1 collegiate athletes. This is the first study to assess the validity of UC within a variety of NCAA Division 1 collegiate athletes. Results from this study emphasized the ability to self-assess hydration status using the UC chart. However, our systematic review determined that the validation of this hydration biomarker in the general adult population is still uncertain.

This dissertation identified the lack of assessment tools to effectively measure beverage intake. Numerous questionnaires have been developed over the past 20 years to better understand beverage consumption within several populations. To date, many of these questionnaires have provided moderate to strong correlations when compared to another dietary assessment reference measures, including food intake records and 24-hr dietary recalls. Further research may be needed to determine which of these validated questionnaires is most optimal for capturing habitual beverage intake and within which populations. This would be valuable in advancing research and assisting healthcare practitioners in educating clients on proper beverage consumption, specifically sugar sweetened beverage (SSB) consumption, for managing weight status.[2] Additionally, many dietary assessment methods have not been evaluated for the sensitivity to detect changes in dietary/beverage consumption over time.[3] Nonetheless, that capability in athletes was not assessed within this dissertation. Further research could assess the ability of the BEVQ-15 to detect the changes in beverage consumption over time within NCAA Division 1 collegiate athletes. This ability will further enhance the research related to nutrition interventions and the responsiveness of the BEVQ-15 within this target population.[4]

While the BEVQ-15 demonstrated validity within this dissertation, modifications might improve the effectiveness of capturing beverage consumption habits within NCAA Division 1 collegiate athletes. Specifically, separating certain categories (i.e. energy/sports drinks and whole milk/2% milk/chocolate milk) could provide a better understanding of the habitual beverage intake and macronutrient consumption. These modifications could also lead to further research re-evaluating the BEVQ-15 within the athletic population. Generalized recommendations for maintaining proper hydration status, in order to optimize athletic performance, have been developed.[5] However, if more categories are added, this could also increase time to complete the questionnaire, which would defeat the purpose of this being a rapid method to assess beverage intake. Therefore, future modifications would need to take this into account. Though, if the modified BEVQ-15 found improvements in the validity of this tool, sports health practitioners could develop individualized hydration and beverage consumption plans for their athletes. These plans could coincide with self-assessment of UC to ultimately improve hydration status, and potentially athletic performance.

In addition, the utilization of UC within the collegiate athletic population presented positive results. However, this dissertation did not utilize a standardized timing protocol for sample collection, due to the need to work within team's practice schedules. Additionally, the athletes' academic schedules need to be taken into consideration when attempting to understand hydration status in any collegiate athletic sport. Unfortunately, at any Division 1 university, it is nearly impossible for every sport to practice and participate in strength and conditioning at the same time every day. However, current literature revolving around the appropriate void collection time (i.e. first morning void, second morning void, or 24hr sample analysis) is difficult to distinguish. Therefore, further research is necessary for determining the best method of assessing hydration status for NCAA Division 1 collegiate athletes.

While extensive research has demonstrated the relevance of maintaining proper euhydration within the athletic population;[6] further research is needed for determining the use of UC and its possible effects on athletic performance. Proper education on the potential result in athletic performance when dehydrated, based on UC, could lead to a reduction in the prevalence of dehydration among athletes.[7] This education intervention could teach athletes how to more effectively self-assess UC and rehydrate appropriately. This is especially important for those athletes exercising in hot and humid environments, such as summer months and specific geographical locations. The prevalence of dehydration is greater among these athletes.[5] Moreover, the validated assessment tools that were used in this dissertation could be utilized with younger athletic populations. Previous research has shown that educational interventions provide significant changes in youth athletic performance when using USG, urine osmolality and UC.[8] Future research could assess athletic performance using the modified BEVQ-15 and the UC chart, through an educational intervention in a variety of younger athletes.

REFERENCES

1. Desbrow B, Jansen S, Barrett A, Leveritt MD, Irwin C. Comparing the rehydration potential of different milk-based drinks to a carbohydrate-electrolyte beverage. *Appl Physiol Nutr Metab.* 2014;39(12):1366-1372.
2. Malik VS, Pan A, Willett WC, Hu FB. Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. *Am J Clin Nutr.* 2013;98(4):1084-1102.
3. Hedrick VE, Comber DL, Ferguson KE, et al. A rapid beverage intake questionnaire can detect changes in beverage intake. *Eating behaviors.* 2013;14(1):90-94.
4. Fok CC, Henry D. Increasing the Sensitivity of Measures to Change. *Prev Sci.* 2015;16(7):978-986.
5. Thomas DT, Erdman KA, Burke LM. American College of Sports Medicine Joint Position Statement: Nutrition and athletic performance. *Med Sci Sports Exerc.* 2016;48(3):543-568.
6. McDermott BP, Anderson SA, Armstrong LE, et al. National Athletic Trainers' Association Position Statement: Fluid replacement for the physically active. *J Athl Train.* 2017;52(9):877-895.
7. Magee PJ, Gallagher AM, McCormack JM. High Prevalence of Dehydration and Inadequate Nutritional Knowledge Among University and Club Level Athletes. *Int J Sport Nutr Exerc Metab.* 2017;27(2):158-168.
8. Kavouras SA, Arnaoutis G, Makrillos M, et al. Educational intervention on water intake improves hydration status and enhances exercise performance in athletic youth. *Scand J Med Sci Sports.* 2012;22(5):684-689.

APPENDIX A. BEVERAGE QUESTIONNAIRE (BEVQ-15)

Beverage Questionnaire (BEVQ-15)

Participant ID: ____________________

Date: ____________________

Instructions:

For the past month, please indicate your intake for each beverage type by marking an "X" in the bubble for "how often" and "how much each time".

1. Indicate how often you drank the following beverages, for example, if you drank 5 glasses of water per week, mark 4-6 times per week.
2. Indicate the approximate amount of beverage you drank each time, for example, if you drank 1 cup of water each time, mark 1 cup under "how much each time". If applicable, indicate the specific type of beverage by marking an "X" in the bubble by the one used (i.e., type of nut milk).
3. When trying to estimate your intake throughout the day, (i.e., water) think about the total amount you drink. For example, 3 times per day and 20 fl oz each time = 60 fl oz per day. If you consume more 60 fl oz per day select "1 time per day" and write the total amount in the last column.
4. Do not count beverages used in cooking or other preparations, such as milk in cereal.
5. Count milk/creamer added to tea and coffee in the tea or coffee with creamer beverage category, NOT in the milk categories; this includes non-dairy creamer. Please indicate the type of creamer and sweetener used by marking an "X" in the bubble by the one used, if applicable.

Type of Beverage	HOW OFTEN (MARK ONE)							HOW MUCH EACH TIME (MARK ONE)					
	Never or less than 1 time per week (go to next beverage)	1 time per week	2-3 times per week	4-6 times per week	1 time per day	2 times per day	3+ times per day	Less than 6 fl oz (¾ cup)	8 fl oz (1 cup)	12 fl oz (1½ cups)	16 fl oz (2 cups)	20 fl oz (2½ cups)	More than 20 fl oz (specify amount below)
Water or unsweetened sparkling water	O	O	O	O	O	O	O	O	O	O	O	O	____
100% Fruit Juice	O	O	O	O	O	O	O	O	O	O	O	O	____
Sweetened Juice Beverage/Drink (fruit punch, juice cocktail, Sunny Delight, Capri Sun)	O	O	O	O	O	O	O	O	O	O	O	O	____
Whole Milk: red cap, Reduced Fat Milk 2%: purple cap, or Chocolate Milk	O	O	O	O	O	O	O	O	O	O	O	O	____
Low Fat 1%: green cap, Fat Free/Skim Milk: light blue cap, Buttermilk or Soy Milk	O	O	O	O	O	O	O	O	O	O	O	O	____
Nut Milk (almond, cashew, coconut) O Flavored, Original, or Plain O Unsweetened	O	O	O	O	O	O	O	O	O	O	O	O	____
Soft Drinks, Regular	O	O	O	O	O	O	O	O	O	O	O	O	____
Energy & Sports Drinks, Regular (Red Bull, Gatorade, Powerade)	O	O	O	O	O	O	O	O	O	O	O	O	____
Diet or Artificially Sweetened Soft Drinks, Energy & Sports Drinks (Diet Coke, Crystal Light, artificially sweetened sparkling water, Sugar-Free or Total Zero Red Bull, Powerade Zero)	O	O	O	O	O	O	O	O	O	O	O	O	____
Sweet Tea (with sugar)	O	O	O	O	O	O	O	O	O	O	O	O	____
Tea or Coffee, black (no creamer or milk) O Sugar, O Artificial Sweetener, O N/A	O	O	O	O	O	O	O	O	O	O	O	O	____
Tea or Coffee (with creamer) O Sugar O Artificial Sweetener O N/A O Milk O Half & Half or Cream O N/A Creamer: O Flavored O Reg. O Sugar-Free O N/A	O	O	O	O	O	O	O	O	O	O	O	O	____
Wine (red or white)	O	O	O	O	O	O	O	O	O	O	O	O	____
Hard Liquor (vodka, rum, tequila, etc.)	O	O	O	O	O	O	O	O	O	O	O	O	____
Beer, Ales, Wine Coolers, Non-alcoholic or Light Beer	O	O	O	O	O	O	O	O	O	O	O	O	____
Other (list):________________	O	O	O	O	O	O	O	O	O	O	O	O	____

Virginia Tech, 2016.

APPENDIX B: INSTITUTIONAL REVIEW BOARD APPROVAL FOR CHAPTER 2

VirginiaTech

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Institutional Review Board
North End Center, Suite 4120, Virginia Tech
300 Turner Street NW
Blacksburg, Virginia 24061
540/231-4606 Fax 540/231-0959
email irb@vt.edu
website http://www.irb.vt.edu

MEMORANDUM

DATE:	June 14, 2017
TO:	Susan E Duncan, Elizabeth Anne Clark, Samantha Bond Kostelnik, Carlin Rafie, Stephanie Grasso
FROM:	Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)
PROTOCOL TITLE:	Functional Beverages
IRB NUMBER:	**17-229**

Effective June 14, 2017, the Virginia Tech Institution Review Board (IRB) Chair, David M Moore, approved the New Application request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

http://www.irb.vt.edu/pages/responsibilities.htm

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As:	**Exempt, under 45 CFR 46.110 category(ies) 2,6**
Protocol Approval Date:	**June 14, 2017**
Protocol Expiration Date:	**N/A**
Continuing Review Due Date*:	**N/A**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

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APPENDIX C. INSTITUTIONAL REVIEW BOARD APPROVAL FOR CHAPTER 3

VirginiaTech

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300 Turner Street NW
Blacksburg, Virginia 24061
540/231-4606 Fax 540/231-0959
email irb@vt.edu
website http://www.irb.vt.edu

MEMORANDUM

DATE:	January 29, 2018
TO:	Kevin Davy, Michelle S Rockwell, Catherine Whitaker Cockrill, Brittany Ryann Thorpe, Brenda Davy
FROM:	Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)
PROTOCOL TITLE:	Validation of Tools to Assess Beverage Intake and Hydration Status in Collegiate Athletes
IRB NUMBER:	**17-048**

Effective January 29, 2018, the Virginia Tech Institution Review Board (IRB) approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

http://www.irb.vt.edu/pages/responsibilities.htm

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As:	**Expedited, under 45 CFR 46.110 category(ies) 3,4,7**
Protocol Approval Date:	**February 2, 2018**
Protocol Expiration Date:	**February 1, 2019**
Continuing Review Due Date*:	**January 18, 2019**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

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Blacksburg, Virginia 24061
540/231-3732 Fax 540/231-0959
email irb@vt.edu
website http://www.irb.vt.edu

MEMORANDUM

DATE: May 25, 2018

TO: Kevin Davy, Michelle S Rockwell, Catherine Whitaker Cockrill, Brittany Ryann Thorpe, Brenda Davy, Samantha Bond Kostelnik

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)

PROTOCOL TITLE: Validation of Tools to Assess Beverage Intake and Hydration Status in Collegiate Athletes

IRB NUMBER: **17-048**

Effective May 25, 2018, the Virginia Tech Institution Review Board (IRB) approved the Amendment request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

http://www.irb.vt.edu/pages/responsibilities.htm

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As:	**Expedited, under 45 CFR 46.110 category(ies) 3,4,7**
Protocol Approval Date:	**February 2, 2018**
Protocol Expiration Date:	**February 1, 2019**
Continuing Review Due Date*:	**January 18, 2019**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

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North End Center, Suite 4120
300 Turner Street NW
Blacksburg, Virginia 24061
540/231-3732 Fax 540/231-0959
email irb@vt.edu
website http://www.irb.vt.edu

MEMORANDUM

DATE:	February 3, 2019
TO:	Brenda Davy, Michelle S Rockwell, Catherine Whitaker Cockrill, Brittany Ryann Thorpe, Brenda Davy, Samantha Bond Kostelnik, Kevin Davy
FROM:	Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)
PROTOCOL TITLE:	Validation of Tools to Assess Beverage Intake and Hydration Status in Collegiate Athletes
IRB NUMBER:	**17-048**

Effective February 3, 2019, the Virginia Tech Institution Review Board (IRB) approved the Amendment request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

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PROTOCOL INFORMATION:

Approved As:	**Expedited, under 45 CFR 46.110 category(ies) 3,4,7**
Protocol Approval Date:	**February 3, 2019**
Protocol Expiration Date:	**February 2, 2020**
Continuing Review Due Date*:	**January 19, 2020**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

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North End Center, Suite 4120
300 Turner Street NW
Blacksburg, Virginia 24061
540/231-3732 Fax 540/231-0959
email irb@vt.edu
website http://www.irb.vt.edu

MEMORANDUM

DATE:	February 5, 2019
TO:	Brenda Davy, Michelle S Rockwell, Samantha Bond Kostelnik, Kevin Davy
FROM:	Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)
PROTOCOL TITLE:	Validation of Tools to Assess Beverage Intake and Hydration Status in Collegiate Athletes
IRB NUMBER:	**17-048**

Effective February 4, 2019, the Virginia Tech Institution Review Board (IRB) approved the Amendment request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

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PROTOCOL INFORMATION:

Approved As:	**Expedited, under 45 CFR 46.110 category(ies) 3,4,7**
Protocol Approval Date:	**February 3, 2019**
Protocol Expiration Date:	**February 2, 2020**
Continuing Review Due Date*:	**January 19, 2020**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

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North End Center, Suite 4120 (MC 0497)
300 Turner Street NW
Blacksburg, Virginia 24061
540/231-3732
irb@vt.edu
http://www.research.vt.edu/sirc/hrpp

MEMORANDUM

DATE:	January 16, 2020
TO:	Brenda Davy, Michelle S Rockwell, Samantha Bond Kostelnik, Kevin Davy
FROM:	Virginia Tech Institutional Review Board (FWA00000572, expires October 29, 2024)
PROTOCOL TITLE:	Validation of Tools to Assess Beverage Intake and Hydration Status in Collegiate Athletes
IRB NUMBER:	**17-048**

Effective January 16, 2020, the Virginia Tech Institution Review Board (IRB) approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

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PROTOCOL INFORMATION:

Approved As:	**Expedited, under 45 CFR 46.110 category(ies) 3,4,7**
Protocol Approval Date:	**February 3, 2020**
Protocol Expiration Date:	**February 2, 2021**
Continuing Review Due Date*:	**January 12, 2021**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

ASSOCIATED FUNDING:

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